Science Progress.

No. 13. MARCH, 1895. Vol. III.

ANTITOXIN.

ONE of the earliest empiricisms with regard to communicable diseases affecting men and animals was the knowledge that some do not affect certain species or certain ages, and, further, that some, like smallpox, scarlet fever, etc., attack an individual generally once only. Medical science has for generations expressed these facts in terms which are familiar to everybody, though their exact nature remained unknown. The terms that were and are still used in a technical sense for indicating that a particular individual is not attackable by a particular disease, are "lack of predisposition," "unsusceptibility," or "immunity". This phraseology went further, and distinguished natural or congenital or spontaneous immunity from "acquired immunity"; the latter indicating that, owing to having passed through one attack, the individual is now possessed of immunity against the particular disease. But it is obvious that these terms are merely paraphrases of conditions that are patent to every one, and are no more or no better understood whether used by laymen or in a technical sense.

After Pasteur showed that various fermentative changes, such as the alcoholic fermentation, acetic acid fermentation, the conversion of urea into ammonium carbonate, are due to the activity of specific microbes—yeast cells, bacterium aceti, or micrococcus ureæ respectively—the infectious diseases were likewise assumed to be comparable to specific fermentative processes, caused in the infected body by the specific microbes; and, further, the nature of natural unsusceptibility and acquired immunity began to be discussed. One of the earliest theories concerning this subject took its basis

from the assumed analogy between fermentative processes and infectious diseases, and stated that, just as in the alcoholic fermentation the yeast cells, that is the specific microbe, require for their life and action the presence of sugar, so also in the infectious diseases the presence in the body of special substances required by the particular microbe is an essential element : without them the microbe cannot live and thrive, and cannot produce the disease.

This theory further assumed, as a necessary corollary, that during the progress of the disease the fermentative substance becomes consumed, and as soon as this stage is reached the disease comes to an end ; as long as the exhaustion of the fermentable substance lasts the body is unsusceptible to further attack. This explanation of acquired immunity is generally spoken of as the exhaustion theory. We know now that this theory is not borne out by direct evidence, moreover it is definitely contradicted by fact. The infectious diseases are not comparable to fermentative changes, such as the alcoholic or acetic fermentation, and we also know that acquired immunity is not due to exhaustion and removal by the specific microbe of some necessary ingredient from the tissues during the first attack.

Klebs first formulated a further theory, which is known as the antitoxin or antidote theory ; this theory explained acquired immunity by saying that during the life and multiplication of the microbes within the infected body—that is, during the attack of the disease—these microbes produce chemical substances which are poisonous to the microbes themselves and their products, so that by the accumulation of these antitoxins in the infected body the disease comes to an end, and reinfection by the same microbes is prevented as long as these antitoxins remain in the body.[1] We shall presently see that recent experimental research has shown that this explanation of acquired immunity is the correct one, although it ought to be stated that the view

[1] In my book, *Micro-organism and Disease*, 3rd edition, 1886, I have in chapter xx. fully considered and supported this theory, and have pointed out that natural immunity and acquired immunity are carefully to be distinguished from one another, a conclusion to which also Buchner arrived within recent years.

formulated by Klebs was purely and simply speculation, for which no direct proof had been furnished by him.

Experimental research has established that the pathogenic microbes produce in the animal body, as also in artificial media of definite composition, poisonous principles —toxins, which differ from one another in physiological action, and which are the immediate cause of the disease symptoms. These toxins have been studied by Hankin, Sydney Martin, Roux and Yersin, Fraenkel and Brieger, Behring and Kitasato, Tizzoni, and others,[1] and these observers have contributed a large amount of exact knowledge as to the specific and different chemical and physiological nature of the toxins produced by different pathogenic bacteria. And although much remains still unknown concerning their chemical nature, a good deal of advance has nevertheless been made already. Thus Roux and Yersin have shown that the toxin produced in broth cultures of the diphtheria bacillus is of the nature of a ferment, which injected as pure toxin (separated from the bacilli) sets up the same typical disease and death as the injection of the bacilli. In the former case the disease is produced as it were at the outset by the injection of the ready-made toxin, in the latter the injected bacilli multiply within the body and therein gradually produce the toxin. Sydney Martin has extended this to a considerable degree, by showing that in the affected human body the toxin (ferment) is actually present, and, further, that this ferment leads to profound changes in certain tissues, whereby albumoses and alkaloids as secondary poisons are generated.

Hankin showed that when anthrax bacilli multiply they produce in the medium in which they grow (fibrine) poi-

[1] Hankin, Anthraxalbumose, *Brit. Med. Journal*, vol. ii., 1890, p. 810.

Sydney Martin, Chemical Pathology of Anthrax, *Report of Med. Off. of Loc. Gov. Board*, 1889-1890; Chemical Pathology of Diphtheria, *Ibid.*, 1890-1891.

Roux and Yersin, Contributions à l'étude de la diphtherie, *Annales de l'Institut Pasteur*, vols. ii., iii., and iv.

Fraenkel and Brieger, *Berl. Klin. Woch.*, Nos. 11 and 12, and No. 49, 1890.

Behring and Kitasato, Diphtherie und Tetanusimmunität, *Deutsche med. Woch.*, 1890, No. 49.

Kitasato, Ueber das Tetanusgift, *Zeitschr. f. Hygiene*, x., 2.

sonous albumoses, while Sydney Martin proved that specific albumoses and alkaloids are produced in the animal body by the multiplication of the bacilli. Kitasato studied the tetanus toxin, and found that it is of a specific nature.

A further important contribution was made by showing that by repeated injections of small non-fatal doses of pure toxin, or of larger doses of weakened toxin (the effect of weakening being caused by the addition of various chemical substances or by heating), an otherwise susceptible animal may be furnished with gradually increasing resistance, not only against fatal doses of toxin but of the bacilli themselves. That a high degree of resistance or tolerance amounting in some cases to immunity can be produced in otherwise susceptible animals by the injection of attenuated living bacilli dates back to the researches of Pasteur on protective inoculations against anthrax and against fowl cholera, and this principle has been applied to various other infectious diseases by many subsequent observers. Now, with regard to tetanus and diphtheria, the observations of Behring and Kitasato, Fraenkel and Brieger, Roux and others have established that by injecting animals repeatedly with gradually increasing amounts of either attenuated culture, or with (non-fatal) small doses of pure toxin, or with larger doses of weakened toxin—produced by the microbe of tetanus or diphtheria respectively in artificial culture—a high state of tolerance can be ultimately achieved whereby these animals become possessed of immunity. And it appears as a further result of these observations, particularly those of Behring and Kitasato, Roux and Vaillard, Roux, (1) that the amount of attenuated culture or of toxin required for each injection stands in indirect proportion to the initial susceptibility of the animal experimented upon, and (2) that the degree of resistance thus acquired is considerable in tetanus and soon reached, while in diphtheria it is reached only slowly, after a large number of preliminary injections, and is at no time of the nature of absolute immunity, though its degree increases as the injections increase in amount and number. Thus, for instance, in Roux's experiments, it is shown that a horse, even after it

had been successively injected a great many times (more than twenty or thirty times) with pure diphtheria toxin, continues to react to every injection as much as at the earlier stages, provided the dose injected be gradually increased.

The most important result, however, of the observations on producing artificial immunity was the one elucidated by Behring [1] and his collaborators; *viz.*, that the blood and blood serum of the immunised animals (diphtheria and tetanus) when injected in certain proportion into otherwise susceptible animals furnishes these latter with immunity, and more than that, is capable of arresting and curing the disease in the already infected animal. These classical experiments of Behring and his collaborators on artificial immunisation, and of using the blood serum of the immunised animals as protective and curative (*Heilserum*) agents (*Serumtherapie*) form the basis of all subsequent observations on antitoxin treatment in diphtheria and tetanus, and without in the least degree wishing to minimise the merits of the contributions of various observers like Babes (in rabies), Tizzoni and Cettani (tetanus), Roux and Martin (diphtheria),[2] it is, I think, admitted that to Behring belongs the credit of having fully grasped the meaning of, and established on a firm experimental basis, the facts concerning artificial immunisation against tetanus and diphtheria, and the use of blood serum of the immunised animals both as protective and curative agents. In addition to this, Behring has clearly established that the blood serum of an immunised animal owes its antitoxic power to chemical substances; that these are present dissolved in the blood, as such and not as cellular bodies; that their action takes place outside (*in vitro*) as well as in the body of an animal; and that, therefore, the production of acquired immunity, *i.e.*, the presence of antitoxin in the blood, has nothing and can have nothing to do with mechanical phagocytosis as advocated by

[1] Behring, Die Blutserumtherapie bei Diphtherie und Tetanus, *Zeitschr. f. Hygiene*, xii., p. 1.

Behring and Knorr, Ueber den Immunisirungswerth u. Heilwerth des Tetanusheilserums bei weissen Mäusen, *Zeitschr. f. Hygiene*, xiii.

[2] Tizzoni and Cettani, *La Rif. med.*, 1893, No. 131.

Roux, *Annales de l'Institut Pasteur*, Septembre, 1894.

Metschnikoff and his pupils. The antitoxin serum acts antagonistically against the toxin *in corpore* or *in vitro* on lines and principles determined by quantity; so that the whole theory of mechanical phagocytosis of Metschnikoff has become superfluous as far as acquired immunity is concerned. When an animal is rendered immune either by repeated injections of attenuated culture or by small (non-fatal) doses of toxin the result is always the same; *viz.*, the appearance in the blood of chemical substances (antitoxin); such a condition of the blood or blood serum becomes established and can be further used both protectively and curatively without any phagocytic interference. In the case of acquired immunity produced by toxin injection, the action of phagocytes, that is, of cells swallowing bacteria, is *a fortiori* out of place, because no bacteria at all are introduced; the curative action of antitoxin serum in tetanus and diphtheria deals with the neutralisation of toxin that had been formed and is circulating in the tissues of the infected body, and therefore also in this the swallowing up and destroying of bacteria has no place.

The manner in which in acquired immunity the antitoxic condition of the blood is brought about is not known. There exist theories only on this subject; thus Buchner inclines to the view that the toxins themselves, produced by the specific bacteria in the infected body, become by the action of the tissues converted into antitoxins; whereas Behring and also Roux ascribe the production of antitoxin to the tissues themselves, these latter being stimulated by the toxins and thus produce or secrete the latter as a sort of self-defence. As a matter of fact in the production of diphtheria antitoxin of gradually-increasing potency in the horse (Roux), this latter has to be injected with gradually increasing quantities of pure toxin in order to produce further reaction in the animal: tumour and rise of temperature. This clearly points to a stimulation of the tissues in the production of the antitoxin.

All who have worked on this subject agree with Behring that the higher the degree of immunisation in diphtheria, that is, the greater the number of injections and the greater the amount injected, the higher the potency of

the antitoxic condition of the blood. This latter is estimated and tested by Behring and also by Roux by the neutralising action it possesses on diphtheria toxin. A toxin is prepared of a given strength—determined by the amount required to kill within thirty hours a given weight of guinea-pigs—of this a tenfold fatal dose is injected into each of a series of guinea-pigs, and subsequently, about six hours later, a definite amount of antitoxin serum is injected into each animal ; that serum which in a given dose is capable of neutralising the toxin, *i.e.*, capable of preserving the animal, is evidently of higher potency than the serum of which a larger dose is required ; or the serum of which a given quantity can neutralise a larger amount of toxin is of higher potency. Now, as regards the application of such standards to the use of the antitoxin serum in the treatment of diphtheria in the human subject, it must at the outset be confessed that there is little to guide us in the matter, for in a case of human diphtheria, in which the serum is to be used, the amount of toxin circulating in the body is an altogether unknown quantity ; whether comparatively or absolutely it is as great or greater than a tenfold fatal dose in the guinea-pigs of a given weight, whether it is greater in a severe case than in a mild case of diphtheria, are matters about which no data exist. All that can be said is that the greater the antitoxic potency of the injected serum, the more likely is it that it will be capable of neutralising the toxin already formed, or likely to be formed, by the still active diphtheria bacilli.

There is one aspect of the subject which requires consideration, and which in the methods of production of antitoxin by Behring and by Roux does not seem to have been sufficiently taken account of. It is this : under natural conditions, when an animal or human being has passed through one attack of an infectious disease and has thereby acquired resistance amounting in some instances to an almost complete immunity, this condition of resistance or immunity is brought about by the activity of the specific microbes ; these during their life and multiplication in the infected body eventually cause the accumulation of the specific antitoxins in the blood and tissues. Now, the

question that presents itself here is this: is this production of immunity due solely to the formation of antitoxin, due to stimulation by the toxins elaborated by the microbes in the body, or is it also due to the presence of substances derived from the microbes themselves? As the disease passes off the microbes themselves degenerate and disappear as such, and subsequent infection of the body with the microbes does not succeed at all, as in complete acquired immunity, or succeeds only partially and after large doses, as in moderate degrees of acquired resistance. It stands to reason that the substance of the degenerating bacterial bodies contributes something towards the acquired refractory state of the blood and tissues, and as a matter of fact it has been shown (Klein, Sobernheim) that the substances of bacteria (intracellular substances) possess in sufficient doses an inhibitory action, inasmuch as they are capable of furnishing the tissues with a refractory power against the subsequent growth and multiplication of the microbes. The assumption, therefore, that the presence of bacterial intracellular substances in the blood and tissues of an animal, after the disease has passed and after the bacteria that caused the disease had degenerated, plays some part in the condition of resistance against a subsequent invasion and multiplication of the specific bacteria, is at least a justified one. Fraenkel showed that in cultures of diphtheria bacilli there are two substances present: one the toxin destroyed by heating to 60°-70° C., and another the immunising substance not destroyed at 60°-70° C.; it is quite feasible to assume that this latter owes its origin to and is identical with the bacterial substances themselves; moreover he showed that if a culture be heated to 60°-70° C. (whereby the toxin is destroyed), and then injected in sufficient quantities subcutaneously into the guinea-pigs, these latter, after the lapse of at least fourteen days, are protected against a second injection with a fatal dose of living bacilli. The lapse of at least fourteen days is very suggestive for the above-expressed view, that the bacteria eventually yield substances which form one of the elements of the immunising action.

When an animal acquires resistance by repeated injections with gradually increasing doses of diphtheria toxin,

its blood becomes charged with antitoxin of gradually increasing potency (Behring, Roux), so that the true antitoxic condition of the blood of such an animal can only be due to the action of that toxin; but an animal is also furnished with resistance by repeated injections of living bacilli in gradually increasing amounts and virulence; in this process antitoxin is formed indirectly by the multiplying and toxin-producing bacilli, but in addition, when the bacilli cease to multiply and become degenerated, immunising substance derived from the bacterial bodies becomes available. The total amount of these latter would determine the amount of immunising substance, and would of course depend on the total amount of bacteria that had been injected in the various operations, just as the amount of antitoxin depends on the amount and degree of potency of the stimulating toxin injected.

Considerations like these have led me to immunise horses against diphtheria by injecting them repeatedly with large doses of living bacilli of increasing virulence, and thereby blood serum has been obtained which when tested on the guinea-pig has a powerful immunising (protective and curative) action on otherwise fatal doses of living bacilli. The serum of such horses possesses, in addition to antitoxin, substances which act powerfully on the living bacilli. True, the antitoxin is not of such potency as can be got by long-continued injection of large doses of pure toxin, as in the animals prepared by Behring and Roux; the immunising action of the serum obtained by the former methods is the conspicuous part, but there is also antitoxin present, since the living bacilli at each injection cause by their multiplication toxin: rise of temperature and tumour. That the presence of immunising substances is important in protective inoculations has been already mentioned; it now only remains to draw attention to the part they play in the curative proceeding, for it must be obvious that the introduction of sufficient amounts of immunising substances—which have been shown to act specifically against the living bacilli—into the system of an animal and man subject to diphtheria, *i.e.*, in which the living bacilli are still multiplying and producing toxin, must materially accelerate the time in which the life of the bacilli is brought to a standstill, and the production of toxin arrested; that is to say, the disease is cut short.

E. KLEIN.

FOREIGN WORK AMONGST THE OLDER ROCKS.

FOREIGN workers amongst the older rocks have written a number of books and papers published during the earlier part of the year 1894. Much of the literature consists of a description of local details, more or less valuable, which will eventually be fitted into their places in the great geological puzzle, though at present they lie unused, as their connection with those parts of the puzzle which have been pieced together cannot be traced. In other papers we find pieces which can be fitted, and in others again evidence of pieces formerly supposed to have been assigned to their right positions which must be removed and placed elsewhere.

The work here noticed is concerned with Precambrian and Palæozoic rocks. The principal task amongst the former rocks is the gradual removal of rock-masses from the group of rocks whose origin is unknown to that whose mode of production is known or inferred. The work in the Palæozoic rocks is instructive. Detailed work again and again proves the value of fossils as means of identifying strata, and clears up difficulties that have arisen from too hasty examination of strata, whilst as a result of rapid work we are furnished with new difficulties, explained away by the handy theories of colonies and migrations. As the laborious worker in a district of a few score square miles breaks down case after case upon which the advocates of these convenient "refuges for the stratigraphic destitute," as they have been termed, rely, the lightning-stratigrapher, traversing half a continent, produces fresh instances, which will in turn doubtless disappear. Whilst alluding to this subject it is satisfactory to call attention to a remark made by Sir William Dawson showing that plants, as well as animals, when properly studied, give no countenance to the existence of anomalous "colonies". He says (1): "Each geological period was sufficiently long to permit plants to

migrate to every station they could occupy, and in every case the temporary and local climate must be indicated by the local flora, while the succession in any one place may be relied on as holding good over a very extensive area". Geologists may be congratulated on having the length of geological periods insisted on by so eminent a writer in connection with this very question, for the reluctance of men of science to accept the contemporaneity of widespread deposits is undoubtedly due to their inability to grasp the idea of geological time as indicated by geological data, and the tendency to take their time from the astronomer and physicist. When premature attempts to speak of geological time in terms of thousands or millions of years are abandoned, so much the better for the science.

In noticing the work done amongst the older rocks abroad, it will be convenient as before to take them in order of antiquity, beginning with the oldest, but, before doing this, attention must be called to certain works treating of deposits of many ages, or bearing upon general questions. As I had occasion in a former article to write at some length upon the discoveries recently made amongst the older rocks of India, it is only necessary to mention that a full account of the older as well as the newer rocks of India appears in the new edition of the *Geology of India* (2), which was received in this country in 1894. The editor devotes a chapter (chap. viii.) to the vexed question of the age of the Gondwana series, and is able to compare the beds of India with those of Africa and Australia.

An interesting paper by M. Marcel Bertrand (3) treats of the Palæozoic and Tertiary synclinal folds affecting the French rocks. This author announced about two years ago the existence of two fundamental laws of the deformation of the earth's crust. First, that the foldings always follow the same lines; and second, that these lines, however varied, form on the whole a right-angled network of parallels and meridians. In the present paper he supports these laws by reference to a geological map of France, showing the trend of the Palæozoic and Tertiary synclinal axes. He also discusses the relation of the distribution and

variations in the characters of sediments to the lines of folding. As will be noted in a future article, the enunciation of these laws is of interest as bearing on the distribution of coal beneath the newer rocks of South-eastern England.

The Carnic Alps are the subject of two memoirs (4, 5), of which the first is a continuation of a part previously published, but the two parts have been issued in book form with some large extra geological maps. Rocks belonging to all systems, from Precambrian to Permian inclusive, are described. The Silurian rocks apparently resemble those of Eastern Bohemia, and above them a great mass of Devonian limestones is developed, separated from the Silurian by a marked change of fauna. The lower part of this limestone possesses a Hercynian fauna, the middle portion contains the fossils of the Middle Devonian (including Stringocephalus), and the upper portion those of the base of the Upper Devonian. Here, as in Bohemia, we have the deep water representatives of the Devonian period. The most interesting feature of this greatly disturbed district is the occurrence of "reef-limestones" with their accompanying breccias, amongst the Devonian and Triassic deposits. An admirable description of them is given in Frech's valuable work, and no one who is interested in these very remarkable, and, at the present time, much discussed structures, must omit to study it.

Proceeding now to consider the other writings in order of age of the rocks described, we will commence with those which treat of:—

A.—PRECAMBRIAN ROCKS.

The student will do well to consult a paper by Van Hise (6), which is a continuation of a former paper, and contains abstracts of papers concerned with the Precambrian rocks of America. The most important contribution to the literature of these ancient rocks published during the period under notice is by Whittle (7). The geology of the area described herein (Vermont) is of special interest, as the region is the home of the Taconic controversy, and when

we remember the almost hopeless state of confusion into which the classification of the older rocks of America was thrown during the raging of this controversy, it is a matter of congratulation to find, as shown by this and other papers to be presently noticed, that the strata of this complicated region have been reduced to order by more detailed work. The author of the present memoir, who notices the region in another paper (8), describes the structure of the Green Mountains as consisting of "a series of sharp compressed folds striking approximately north and south, and overturned to the west in most localities, so that induced schistosity and stratification dip eastward". He uses the now familiar term "Algonkian" for those Precambrian rocks which lie above the "Archæan" rocks, and separates them into two series, the upper or Mendon (which underlies the Olenellus quartzite), and the lower or Mount Holly series. As the uppermost rocks of the Mendon series consist of mica schists succeeded with apparent conformity by the quartzites, "it must be left for future work to determine beyond dispute the relations of the series immediately below the Olenellus zone to the quartzite, whether the rocks are conformable or unconformable; if the former, whether the delimitation of the Lower Cambrian shall be placed above the mica schist or below it. Tentatively, the series just below the quartzite, the mica schist at the top, and the conglomerate at the bottom, will be considered wholly or in part of Algonkian age." The variations in the characters of the Mendon series are mainly of interest to the petrographer, but where least altered, their clastic character is evident, and sandstones and conglomerates occur. In the typical section the series is about 1300 feet thick, but in the northern part of the State it is much more developed.

The core of the Green Mountains is composed of rocks belonging to the Mount Holly series, a more variable and more metamorphosed set of stratified rocks than those of the Mendon series, but "a description of the different consecutive members of the series cannot be given, as the rocks are too variable in character, and dynamic action has involved them in great complications". Amongst the

altered sediments are gneisses and schists of unknown origin, and "abundant metamorphic equivalents of old basic igneous rocks" now in the condition of amphibolites.

The paper contains descriptions of the conversion of clastic rocks into schist and gneiss, including a stretched "conglomerate gneiss" of which a figure is given, recalling in a marked degree some specimens of the Obermittweida Conglomerate of Saxony and certain Norwegian conglomerates described by Reusch.

In a paper by Ll. Smith (9) the relationship of certain Precambrian iron-ore and Jasper-bearing rocks above the "Archæan" rocks is discussed.

Since the days of the discovery of Eozöon Canadense the search for Precambrian fossils has been zealously prosecuted, but hitherto with unsatisfactory results, and although we are prepared any day to hear of the actual finding of determinable Precambrian organisms, it is by no means certain that they have yet turned up. These remarks are suggested by a paper (10) announcing the discovery of foraminifera in the Precambrian rocks of France. Tests of these organisms are stated to be contained in quartzites and phtanites interstratified with Precambrian phyllades of Saint-Lô (Côtes-du-Nord), in which the existence of radiolaria had previously been announced. The age of the rocks does not appear to have been disputed, but judging from the cautious way in which the supposed organisms are spoken of in a review in the *Geological Magazine* for 1894, p. 417, which gives evidence of having been written by a master of the subject, their organic nature cannot be considered established beyond doubt, and we still await the description of a fauna older than that of the Olenellus beds.

B.—LOWER PALÆOZOIC ROCKS.

Two papers by Walcott (11) give additional information concerning the Olenellus beds, and further clear up the difficulties which have arisen around the "Taconic Question". The correctness of the early correlations of McClure, Eaton, Emmons and Rogers is established. Recent discoveries show that the basal sandstones of Alabama,

Tennessee, Virginia, Maryland, Pennsylvania, New Jersey, New York and Vermont were all deposited in Lower Cambrian times, and contain the Olenellus fauna. The overlying limestones have the Olenellus fauna in their lower portions in Vermont, Eastern New York, New Jersey and Pennsylvania. It is interesting to find that these limestones contain faunas of ages up to and including the Trenton. It is not clear at present where the line between Cambrian and Ordovician should be drawn in these limestones. The existence of many faunas in this accumulation of clear water limestones is of particular interest to British geologists when it is remembered that the Durness Limestone of the North-west Highlands of Scotland is generally looked upon as if belonging to the American type. Attention may be drawn to the adoption of the term Ordovician by Walcott, one of the principal American authorities on the Lower Palæozoic rocks. Its universal use seems to be but a question of time.

A paper by Andersson (12) is of interest as bearing on the classification of the Lower Palæozoic beds of Scandinavia. He describes the fossils found in loose blocks on the south-east of the island of Öland, and places the beds which have yielded the blocks in the following ascending order: *Macruruskalk*, grey *Trinucleus* Shales, red *Trinucleus* Shales and Limestones, and Limestones with *Leptæna Schmidti*, Törnq, including all in the Lower Silurian (Ordovician). The position of the *Leptæna* limestone has been disputed, but the accumulation of evidence all points to the correctness of Andersson's grouping.

A large number of papers bearing on the Lower Palæozoic rocks are purely palæontological, many of them referring to the trilobites (13), and it will be noted that additional information concerning the appendages of these creatures is forthcoming. Simultaneously, our knowledge of the minute structure of the graptolites is increasing; Wiman (14) describes the nature of certain Diplograptids from a fragment of the "Ostseekalk" of Bornholm, discussing the relations of the sicula, virgula, and hydrothecæ to one another, whilst all geologists will welcome Jaroslav

Perner's first contribution towards the history of the Bohemian graptolites (15). This work is a continuation of Barrande's great work on the Bohemian Palæozoic fauna, and the appearance of the first part containing a description of the structure of *Monograptus* and *Retiolites* illustrated by admirable plates augurs well for the excellence of the remainder of the work on the graptolites of this classic region.

C.—DEVONIAN ROCKS.

Work on the Devonian rocks has been published in both Old and New Worlds, and the subjects discussed in the papers are of varied interest.

Amongst the stratigraphical papers is one by Penecke (16) which commences with an account of the literature and a historical sketch of the Devonian of the area. The beds rest on Neritenschiefer and Crinoidenkalk of (Upper) Silurian age, and the following classification is given in ascending order :—

Quartzitstufe (= F. of Bohemia). . .	Lower Devonian.
Barrandei-schichten (with *Heliolites Barrandei*).	
Kalkschiefer der Hubenhalt (= *Cultrijugatus*-schichten?)	Middle Devonian.
Calceola-schichten	
Limestone with *Cyathophyllum quadrigeminum*	
Goldf. Hochlautschkalk (= Stringocephalenkalk).	

Gap.

Clymenienkalk. . . . . Upper Devonian.

The author gives fossil lists from the different fossiliferous horizons, and a description of new species.

Devonian rocks are also described as occurring in the Isle of Elba (17). Fossils were found in 1892 in the "schistes lustrés" of the east coast of the island. The species are mostly new, but the general character of the fauna, which includes *Hyolithes*, *Beyrichia*, *Plumulites*, *Goniatites*, *Bellerophon*, *Anthracoptera*, *Myalina*, etc., indicates that the rocks are Devonian, and probably Lower Devonian.

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The Palæozoic rocks of Tuscany can now be divided into :—

1. Middle Silurian with *Gomphoceras*, *Cardiola*, etc.
2. Lower Devonian of Elba.
3. Upper Coal Measures of Monte Pisano, etc.

I have been unable to obtain access to another paper (18) on Devonian rocks occurring at no great distance from these.

Follenius (19) describes a coal-seam occurring in the Upper Devonian (Coblenzian) of the Eifel district. It contains remains of marine plants—*Haliserites*—described as generically identical with *Psilophyton* of the American Devonian rocks and with *Hostinella* (*Fucoides*) *hostinensis* of the *Étage H.* of Bohemia. The Bohemian beds *H.* are now generally admitted to be Devonian, and indeed are referred by Frech to the Upper Devonian, and it is interesting to find remains of this plant so widely spread in beds of this age. Another remarkable discovery of similar organisms in beds of about the same age in remote regions is seen in the case of *Spiraxis*. Stainier (20) has discovered a new form belonging to this genus in the Psammites du Condroz. The genus was originally described by Newberry from the Chemung rocks of North America, of an age about equal to that of the Condrusian deposits. Our present author is inclined to regard these remarkable bodies as algæ.

In the New World the discovery of Devonian rocks in California is announced by Diller and Schuchert (21). Dr. C. A. White originally suggested that parts of the auriferous states were older than the Carboniferous. Since then Silurian rocks have been discovered in the region, and now the authors bring forward evidence of the existence of beds belonging to the Middle Devonian (probably its lower part), about the horizon of the Corniferous group, but the correlation is somewhat doubtful, as the fossils found are chiefly corals, whose vertical distribution has not yet been systematically worked out.

D.—CARBONIFEROUS ROCKS.

We find many additions to our knowledge of the carboniferous deposits of France and Belgium. Julien (22) gives reasons for concluding that the relative ages of the beds of the two coal basins of Commentry and Saint Etienne can be fixed by a comparison of their floras. The flora of Commentry contains 210 species, and is divisible into minor floras at different horizons. The strata of the Commentry basin to the east are subdivided into three groups by the intercalation of a very fossiliferous sandstone, the "banc des Roseaux," and a little higher up by another deposit, an unfossiliferous "breccia," the "banc des Chavais," which the author considers to be of glacial origin. To the west the deposits lose their unity of character, and are divisible into six groups separated by shale-partings. The "banc des Chavais" disappears, as does also the "banc des Roseaux," but the latter is replaced by an equally fossiliferous deposit, the "banc de l'Ouest"; at the base a grit separates the main mass of the strata from the "glacial" breccia of Sainte-Aline.

Examining each minor flora separately, noting the species common to the Saint Etienne and Commentry basins, and having studied their exact distribution, the author is enabled to establish the synchronism of the corresponding subdivisions in the two basins. He gives a brief account of his results, reserving details for a further communication. He maintains that the glacial origin of the various breccias of Commentry, established by a study of their character, is confirmed by the palæontological stratigraphy.

Stainier (23) and Briart (24) give accounts of the coal basins of Andenne and Hainault. The former basin constitutes a natural division of the large Liège basin, and its structure is analogous to that of the larger basin. The paper gives details concerning the stratigraphy and disturbances of the rocks of the basin. A reversed fault, the "faille de Boussale," brings Devonian rocks over coal measures at the south-east end of the basin; it gradually dies out towards the west. The Hainault basin is also

affected by considerable disturbances, described in Briart's paper. In the volume of the *Annals of the Geological Society of Belgium*, which contains the latter paper, are several short papers on Palæozoic rocks, including one by Briart on the geology of the neighbourhood of Fontaine-l'Evêque and of Landelies, which gives details of the subdivisions of the remarkable mass of Carboniferous limestone occurring near the latter place.

An extremely interesting contribution to our knowledge of the Carboniferous rocks is by Nathorst (25), in which the Palæozoic floras of Spitzbergen, Bear Island and Nova Zembla are described.

The Liefde Bay beds of Spitzbergen were formerly considered to be passage beds between Devonian and Carboniferous. They are now definitely referred to the Devonian; a new flora has been discovered in them containing *Lepidodendron*, *Bergeria*, *Bothrodendron* and *Psygmophyllum*. The coal flora is described, and the beds containing it referred to the Lower Carboniferous, the flora being compared with that of the Calciferous Sandstone.

A description of the plants of the "Ursa-stage" of Bear Island is given, and this stage referred to a position intermediate between the Lower Carboniferous and the Devonian.

In Nova Zembla certain plants have been discovered whose horizon is not settled.

To stratigraphical geologists the Spitzbergen geology is specially interesting, on account of the former belief in the anomalous distribution of the fossils (see Nordenskjöld, *Geol. Mag.*, 1876, p. 63). The beds containing the coal flora were formerly believed to occur above the mountain limestone proper, though the fauna of this limestone showed remarkable Permian affinities, containing such forms as *Camarophoria humbletonensis*, *Productus cancrini*, *P. horridus* and *Strophalosia lamellosa*. It is now found that the apparent succession is due to inversion, the older plant-bearing beds being brought over the newer limestones. These limestones are not of mountain limestone age, but

representatives of the now well-known Permo-Carboniferous limestones, developed in India and elsewhere. We have therefore in Spitzbergen:—

1. Hekla Hook Beds = Silurian.
2. Liefde Bay Beds = Devonian.
3. Ursa-stage = Passage between Devonian and Carboniferous.
4. Coal-bearing Strata = Lower Carboniferous.
5. Limestones = Permo-Carboniferous.

The mountain limestone proper, which should occur between 4 and 5, has not been detected, but it is suggested that it is possibly represented by the *Cyathophyllum* limestone.

The Marine Upper Carboniferous beds of Arkansas are the subject of a paper by J. P. Smith (26). He states that they correspond to the lower part of the Cisco beds of Texas, and belong to the top of the true Carboniferous, and are below the transitional Permo-Carboniferous. He remarks that Waagen's comparative table showing the relationship of the Upper Palæozoic strata all over the world is not generally accepted by most American geologists as regards the position assigned to some American deposits. Whilst this paper shows the gradual acceptance of the existence of marine coal measures and Permo-Carboniferous faunas (with which we in Britain are practically unacquainted), certain apparent abnormalities are touched on, and the author remarks that they have been explained by some writers as explicable upon the theory of Colonies. This explanation he seems to look upon with approval. The difficulties will no doubt be cleared up ultimately, and, perhaps, when that is done, Waagen's correlations will be proved to hold good for America also.

No important papers have been published concerning the Permian rocks proper during the six months under review.

BIBLIOGRAPHY.

(1) DAWSON, SIR W. The Fossil Plants of Canada as Tests of Climate and Age. *Natural Science*, vol. iv., p. 177. London.

(2) MEDLICOTT, H., and BLANFORD, W. T. *A Manual of the Geology of India*, second edition, edited by R. D. Oldham. Calcutta, 1893.

(3) BERTRAND, M. Lignes directrices de la geologie de la France. *Comptes Rendus Acad. Sci. Paris*, p. 258, 1894.

(4) FRECH, F. Die Karnischen Alpen. *Abhand. Naturf. Gesell. Halle*, bd. xviii., heft 2-4, p. 161, 1824.

(5) GEYER, G. F. Zur Stratigraphie der Palæozoischen Schictser in den Karnischen Alpen. *Verh. K. K. Geol. Reichs.*, p. 102, 1894.

(6) VAN HISE, C. R. Summary of Precambrian North American Literature. *Journ. of Geology*, vol. ii., No. 4. Chicago, 1894.

(7) WHITTLE, C. L. The Occurrence of Algonkian Rocks in Vermont, and the Evidence for their Subdivision. *Journ. of Geology*, vol. ii., No. 4, p. 396.

(8) WHITTLE, C. L. The General Structure of the Main Axis of the Green Mountains. *American Journ. of Science*, ser. 3, vol. xlvii., p. 347.

(9) SMITH, H. LL. Relations of the Lower Menominee and Lower Marquette Series in Michigan (Preliminary). *Amer. Journ. of Science*, ser. 3, vol. xlvii., p. 216.

(10) CAYEUX, L. Sur la présence de restes de Foraminifèrés dans les terrains Précambriens de Bretagne. *Comptes Rendus Acad. Sci. Paris*, tome 98, p. 1433.

(11) WALCOTT, C. D. Notes on the Cambrian Rocks of Pennsylvania, from the Susquehanna to the Delaware. *Amer. Journ. of Science*, ser. 3, vol. xlvii., p. 37. Occurrence of Olenellus in the Green Pond Mountain Series of Northern New Jersey, with a note on the Conglomerates. *Ibid.*, p. 309.

(12) ANDERSSON, J. G. Ueber Blocke aus dem Jüngeren Untersilur auf der Insel Öland vorkommend. *Overs. K. Lv. Vet. Akad. Forhande*, 50° *Arg.*, p. 521. Stockholm.

(13) ETHERIDGE, R., JUN. The largest Australian Trilobite hitherto discovered. *Proc. R. Soc. Victoria, N.S.*, vol. vi., p. 189. Melbourne.

ETHERIDGE, JUN., and MITCHELL. On Silurian Trilobites. *Proc. Linn. Soc. N. S. W.*, vol. xiii., part ii., p. 169.

BEECHER, C. E. Appendages of the Pygodium of *Triarthrus*. *Amer. Journ. of Science*, ser. 3, vol. xlvii., p. 298.

WALCOTT, C. D. Notes on some Appendages of the Trilobites. *Geol. Mag.*, Dec., 4, vol. i., p. 246.

(14) WIMAN, C. On *Diplograptidæ*, Lapworth. *Journ. of Geol.*, vol. ii., p. 267. Chicago.

(15) PERNER, J. Études sur les graptolites de Bohême.

(16) PENECKE, K. A. Das Grazer Devon. *Jahrb. K. K. Geol. Reichs.*, vol. xliii., heft 3-4.

(17) STEFANI, C. DE. Decouverte d'une faune paléozoïque a l'Ile d'Elbe. *Bull. Soc. Geol. France*, ser. 3, tome xxii., p. 30.

(18) LOVISATO, D. Il Devoniano nel Gerrei (Sardegna). *Rendiconti Atti R. Accad. Lincei Rome*, ser. 5, vol. iii., p. 131.

(19) FOLLENIUS. Ueber die Kohlenfunde in der Eifel. *Verh. Naturh. verein d. preuss Rheinlande*, Jahrg. 50, Korrespondenzblatt, p. 40 (followed by a note on the plants by H. Pohlig, p. 44). Bonn.

(20) STAINIER, X. Un Spiraxis nouveau du Devonien Belge. *Bull. Soc. Geol. Belg.*, etc., tome viii., p. 23.

(21) DILLER, J. S., and SCHUCHERT, C. Discovery of Devonian Rocks in California. *Amer. Journ. of Science*, ser. 3, vol. xlvii., p. 416.

(22) JULIEN, A. Sur le synchronisme des bassins houillers de Commentry et de Sainte Étienne. *Comptes Rendus Acad. Sci. Paris*, tome 98, p. 155.

(23) STAINIER, X. Étude sur le Bassin Houiller d'Andenne. *Bull. Soc. Geol. Belg.*, tome viii., p. 3.

(24) BRIART, A. Étude sur la structure du Bassin Houiller du Hainault. *Ann. Soc. Geol. Belg.*, tome xxi., p. 125.

(25) NATHORST, A. G. *Zur fossilen Flora der Polar länder*, theil 1, lief 1. Zur paläozoischen Flora der Arktischen Zone. *K. Vetensk. Akad. Handlingar*, bd. xxvi. Stockholm.

(26) SMITH, J. P. The Arkansas Coal Measures in their relation to the Pacific Carboniferous Province. *Journ. of Geol.*, vol. ii., p. 183. Chicago.

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INSULAR FLORAS.

PART IV.

FROM the main Atlantic we may return northward, diverging to the West Indies. Much has been done during the past ten years in the botanical exploration of the smaller islands of the West Indies, chiefly under the auspices of the British Association for the Advancement of Science, supported by grants from the Royal Society. The Germans have also taken up the matter, and Dr. I. Urban, of Berlin, has in hand a flora of all the islands. The Committee appointed by the British Association held its first meeting in January, 1888, and steps were at once taken for carrying its recommendations into effect. A report containing a botanical and zoological bibliography (1) of the chain of islands from Tobago to Porto Rico was issued by the association the same year, followed by annual reports up to the present time. Mr. F. Du Cane Godman assisted materially by the employment of private collectors, both in botany and zoology. The bibliography referred to above contains not only the titles of the books and articles treating of the botany and zoology of the islands, but also the main facts then known relating to each island or group of islands, it having been intended as a kind of guide for the use of collectors and elaborators.

Nearly all of the British Islands, from Tobago to the Virgin group, were more or less thoroughly explored, the staff of the various botanical stations in the West Indies having worked in concert with the special collectors; and the results have been published, some in detail, some in summary only.

It may be premised that the flora of the whole chain of islands is very uniform in composition throughout; therefore it will be sufficient to discuss two or three of the more important contributions, though, for convenience, references are also given to the smaller ones. The islands may be taken in order, from south to north, beginning with

Grenada. Several of the islands are remarkably rich in ferns, notably Jamaica, where, including lycopods and other vascular cryptogams, there are probably not fewer than 500 species. A descriptive account of them is appearing (2) by driblets, and at the present rate will not be completed for some time.

Under the direction of the Committee named above, Mr. Sherring botanised the relatively small island of Grenada during the winter of 1890-91, and devoted himself specially to the fern vegetation. He succeeded in collecting 145 species, an account of which is given (3) by Mr. J. G. Baker. From this we learn that only two species were new, namely, *Alsophila Elliottii*, a tree fern, and *Acrostichum Sherringii*. *Hypoderris Brownii* was known before only from Trinidad, and *Schizæa fluminensis* and *Acrostichum Aubertii* had previously been collected only in Continental America. A few other very rare species were included in the collection; but most of the species are generally diffused in the West Indies. The ferns of Grenada are largely concentrated in the luxuriant forests immediately surrounding the Grand Étang, situated at an elevation of 1740 feet above sea-level. Here about sixty species were observed in a single day. In addition to ferns Mr. Sherring collected about 160 species of flowering plants (4), but as they have not been specially worked out, we may pass on to St. Vincent.

This island was very thoroughly botanised by Mr. Herbert Smith, Mr. Godman's collector, and the plants worked out at Kew (5) in relation to their distribution. I propose drawing freely on this report, in illustration of the botanical geography of the West Indies, because I had something to do with its compilation. St. Vincent lies between Grenada and Santa Lucia; is about eighteen miles long by eleven broad, or half the area of Middlesex; and has a central densely wooded mountain ridge varying from 2000 to 4000 feet in height. Less than one-sixth is under permanent cultivation, and much of the forest is virgin. The smaller islands included in the enumeration referred to are: Bequia, Cannouan, Mustique

and Union, having a total area of less than 9000 acres. With but few and unimportant exceptions, the plants collected in these smaller islands also inhabit St. Vincent. Altogether about 1150 vascular plants have been recorded from the islands, though doubtless a number of them erroneously, as may be inferred from what is known of their general distribution. Mr. Smith collected 977 species, of which it is estimated 131 are colonists, leaving 846 indigenous species. These are referred to 490 genera and 109 natural orders, or rather more than half the orders of the world. This flora being typical of the whole chain of islands, as limited above, it may be worth while giving somewhat full particulars of its composition, relationships and distribution. The twelve predominating natural orders are: Leguminosæ, 141 species; Gramineæ, 58; Rubiaceæ, 52; Compositæ, 45; Orchideæ, 43; Euphorbiaceæ, 38; Cyperaceæ, 34; Melastomaceæ, 25; Malvaceæ, 24; Convolvulaceæ, 23; Solanaceæ, 22; and Piperaceæ, 22. Comparing the foregoing with Baron Eggers's figures (6) for St. Croix and the Virgin Islands, at the other end of the chain, it is not a little surprising to find that the twelve predominating orders are the very same, and follow in exactly the same sequence! Such an approach to uniformity is rare even in unbroken stretches of land at points so distant from one another; much less would it have been expected in islands separated from each other by considerable expanses of water. The fact seems to indicate no very remote period when there was continuity of land.

Pursuing these comparisons further, among flowering plants *Ipomœa* is the most numerous in species in both groups of islands; and such other genera as *Cassia*, *Solanum*, *Panicum* and *Paspalum* are strongly represented in both groups. With regard to the endemic element, it is similar in character at both extremities of the chain. The following species have hitherto only been met with in St. Vincent, but there is no endemic genus: *Trigynæa antillana* (Anonaceæ), *Spachea perforata* (Malpighiaceæ), *Meliosma Herbertii* (Sabiaceæ), *Calliandra Guildingii* (Leguminosæ), *Psidium Guildingianum* and *Gustavia antillana* (Myrtaceæ),

Tibouchina cistoides (Melastomaceæ), *Begonia rotundifolia* (Begoniaceæ), *Hoffmannia tubiflora* (Rubiaceæ), *Malouetia retroflexa* (Apocynaceæ), *Columnea speciosa* (Gesneraceæ), *Peperomia cuneata* and *P. vincentiana* (Piperaceæ), *Croton Guildingii* (Euphorbiaceæ), *Epidendrum vincentinum* (Orchideæ) and *Tillandsia megastachya* (Bromeliaceæ). Assuming that the foregoing species are all actually confined to St. Vincent, the endemic element there is greater than in any other island in the chain; but a thorough working out of the materials from all the islands would probably reduce this number. It would appear, too, that there is a process of extinction going on, for certain plants gathered in St. Vincent in the middle of the present century have not been found since, either there or elsewhere. Such are *Spachea perforata* (Malpighiaceæ) and *Cyathea tenera*, a tree fern. Of course some of these plants may be exceedingly local and still in existence. Now, taking the whole vascular flora of St. Vincent, and following the extensions of the species, we find that out of the 846 inhabiting St. Vincent. 565 are Continental American, most of them having a wide range therein; but few of them extending to the Old World or to Polynesia. Taking those of wider areas, 29 are common to Africa and America, and 133 are of wider extension, many of them being almost cosmopolitan in the tropics. It should be borne in mind that most of the two last categories are also Continental American as well as West Indian. It should also be understood that all of these figures are no more than rough approximations. Coming to narrower areas, or smaller extensions, 198 species are, so far as our present knowledge goes, restricted to the West Indies, and eight others extend only into the Keys of Florida. I have something more to say presently concerning the tropical element in South Florida.

There is still one more class of plants to mention, namely, such as are next in degree of rarity to the endemic species, occurring only in one or two other islands besides St. Vincent. These plants might perhaps be appropriately designated the dying out element. Examples of this class are: *Brownea speciosa*, *Combretum eriopetalum*, *Couroupita*

antillana, *Aspidosperma sessiliflorum*, *Solanum hirtum* and *Pilea ovalis*—St. Vincent and Trinidad; *Voyria tenella*, *Solanum retrofractum* and *Tillandsia compressa*—St. Vincent and Jamaica; *Triumfetta grossulariæfolia*, *Erithalis angustifolia* and *Eupatorium Ossæanum*—St. Vincent and Cuba; *Henriettella triflora*—St. Vincent and St. Lucia; *Begonia dominicalis* and *Stelis scabrida*—St. Vincent and Dominica; *Begonia martinicensis* and *Pleurothallis floribunda*—St. Vincent and Martinique; and *Echites circinalis*—St. Vincent and Hayti. As already intimated, however, too much weight must not be attached to these data, because further researches may more or less invalidate them; yet with this qualification they may be accepted as facts for discussion.

Comparing the number of species of the St. Vincent Flora restricted to the West Indies with Grisebach's estimate (7) for the whole of the West Indies, we meet with a great discrepancy. He came to the conclusion that nearly 50 per cent. of the species that had come under his observation were peculiar to the islands, whereas the components of the St. Vincent Flora include less than 25 per cent. of no wider range than the West Indies. It is true there is a very large endemic element in Cuba, and a considerable one in Hayti and Jamaica, but later discoveries have certainly reduced the proportions he estimated. With regard to the number of species confined to each of the principal islands of the chain of islands under discussion, Grisebach's figures are something lower than is actually the case. He enumerates twenty-nine as peculiar to Dominica, probably owing to the absence of material for comparison. For the others his figures are: St. Vincent, 12; Montserrat, 2; Grenada, 2; Martinique, 2; Guadeloupe, 1; St. Lucia, 1; Antigua, 1; and Barbadoes, 1. Further investigations have added almost nothing.

As already mentioned, most of the other British Islands have been botanised by the agents of the Committee, and the results are briefly recorded in the annual reports, but they contain nothing of sufficient interest for reproduction here. Baron Eggers has published (8) a supplement to

his *Flora*, containing additions and corrections, but no discovery of importance is noted, except the curious new genus, *Eggersia buxifolia* (Nyctagineæ), previously published elsewhere (9), and first discovered by the author in the island of St. Thomas. It is a small apparently evergreen shrub with small leaves and exceedingly small flowers, singularly unlike anything else in the order.

Continuing in a westward direction we next come to Porto Rico.

There is an unfinished descriptive work on the Flora of Porto Rico, written in Spanish (10); and, as no continuation has appeared, so far as I can ascertain, since 1888, it is not probable that it will ever be completed. It includes the natural orders Ranunculaceæ to Gesneraceæ, and is the work of an enthusiastic amateur, and doubtless serviceable on the spot, though not so critically worked out as might be wished. Another Spanish work (11) should be mentioned, although of somewhat earlier date, because it contains descriptions of two or three proposed new genera and some sixty species, though, truth to say, it is even less critical than the preceding. Any plant the author was unable to identify with a published description or figure he described on his own account; consequently it is not worth while repeating his names here. Some of the plants were verified by Dr. Kränzlin and others, at Berlin, notably the orchids and ferns, three of the former being described as new. The proposed new genera are: *Stahlia* (Leguminosæ), *Homonoma* (Melastomaceæ) and *Atelandra* (Myrsineæ).

Hayti, or San Domingo, is the least known botanically of the larger West Indian Islands, yet it was the scene of Father Plumier's labours at the end of the seventeenth century and the beginning of the eighteenth. The unsettled condition of the inhabitants has doubtless had something to do with this. Several botanists, however, have collected in the island within the last ten years, but no consolidated account of the results of their labours has appeared. Baron Eggers visited the island in 1887, and made considerable collections; and he gave an account (12)

of his journey with some general remarks on the vegetation, but he reserved the botany for a separate article, which, I believe, has not yet been published. Nevertheless, the article in question contains many facts of great interest. Pine forests (*Pinus occidentalis*) of large extent exist in the uplands, with an undergrowth of the common bracken (*Pteris aquilina*) in some parts, associated with species of *Fuchsia*, *Hypericum*, *Siphocampylos* and *Pentarraphia*. The pines support a variety of epiphytes and parasites, including Bromeliaceæ, Piperaceæ, Orchideæ, Aroideæ, etc. San Domingo is the only island of the West Indies in which the genus *Fuchsia* is known with certainty to exist, though there is a record which may refer to Cuba. *F. triphylla*, the species on which the genus was founded, was rediscovered some years ago (13) and brought into cultivation. Baron Eggers collected it, and also discovered a very handsome new species (*F. Pringsheimii*, Urban). Here, as in Mexico, scattered palms are found in the pine forests, affording a very striking contrast. Eggers specially mentions a species of *Euterpe* occurring among the pines in San Domingo.

Another tree of more than ordinary interest is the native walnut (*Juglans jamaicensis*), which inhabits the valley of Jarabacoa, and is described as having a pointed fruit. *Juglans jamaicensis* was first described by C. de Candolle (14) from a coloured figure (15), where it is recorded as a native of Jamaica, inhabiting the mountains, and enjoying a high reputation for medicinal virtues. The date of this record is 1829, but I have found no other mention of its occurrence in Jamaica, and there are no specimens in the Kew Herbarium. Yet the island has probably been more thoroughly explored botanically than any of the other large islands. *Juglans insularis* is a native of Cuba, the only other island besides Porto Rico and San Domingo known with certainty to possess a walnut. The specimens from these islands have been named *J. jamaicensis*, though, from the foliage alone, I should have taken them to belong to the same species as the Cuban; but Dr. Urban writes that there are differences in the fruit. No complete enu-

meration of the plants collected by Eggers has appeared, but an uncritically compiled list is given (16) by Tippenhauer. This I have not seen, and only know of its existence from a short review (17), where it is stated that 100 pages are devoted to botany, and the number of plants enumerated reaches 3193, brought together from a variety of sources.

Two enumerations of the plants of the Bahamas have appeared (18 and 19), neither of which can lay claim to completeness; but between them they are sufficient to give a correct idea of the character and composition of the flora, and probably include very nearly all the flowering plants of the whole group of islands. The first, however, is a compilation from several sources, and by several persons of very unequal qualifications for the task. As the geographical part is perhaps the weakest, I may pass on to the second, though I should add with regard to the first that the names were largely contributed by Kew, based on specimens collected by Mr. Brace, and may be taken as approximately correct. Mr. Hitchcock's paper deals more especially with the geographical questions, and it is supplemented by a tabulated view of the distribution of the plants collected in the Bahamas, within the islands of New Providence, Eleuthera, Cat, Watling, Crooked, Fortune and Inagua, and their extensions to Cuba, Central America, South America, Virgin Islands and South Florida. The islands of this archipelago are very numerous, but the largest of those enumerated above, New Providence, is only twenty-one miles by seven, and none of them rises more than 300 feet above the sea-level; consequently there is even less variety in the vegetation than in the Lesser Antilles. Indeed the flora is almost wholly maritime in character; yet a few species have been collected in these islands that have not hitherto been found elsewhere. It is also at the same time essentially Cuban in character, as 321 out of 380 species in Mr. Hitchcock's Bahama collection are known to occur in Cuba. As many as 129 reach South Florida, but these are all southern types, which have their northern limit in Florida or the Southern United States.

On the other hand, there are no essentially northern types extending southward to the Bahamas. The only exception is *Distichlis spicata* (Gramineæ), which has a wide range in North America, and occurs isolated in the island of Inagua.

Out of sixty-eight species common to the Southern United States and the Bahamas, fifty-eight inhabit Cuba. Hitchcock's table does not show how many of the species are peculiar to the West Indies, but he enumerates thirteen as peculiar to the Bahamas. Specially noteworthy among these is *Pinus bahamensis*, apparently confined to the islands of New Providence and Andros, according to Eggers (20), but I have seen no specimens from the latter island. On the authority of Gardiner (18) this pine occurs in the islands of Abaco, Bahama, Berry, New Providence, and the northern half of Andros. It is a very distinct species with leaves a foot long, in clusters of threes.

A curious feature in the West Indian maritime flora deserves mention here, for both Eggers and Hitchcock refer to it, and I have seen specimens from the Bermudas exhibiting the same peculiarity. It is the existence of hairy and glabrous varieties of the same species growing intermixed. *Conocarpus erecta* (Combretaceæ), *Borrichia arborescens* and *B. frutescens* (Compositæ) furnish striking examples of this phenomenon. Some individuals have perfectly glabrous leaves, whilst in others the leaves are densely clothed with a silky, silvery tomentum. Occasionally the same individual bears both kinds of leaves. Baron Eggers's Bahama collection has not been worked out in detail, as it contained little that was new; but some interesting facts illustrated by it have been published (21). Mr. Baker there estimates the total number of species known from the whole group of islands at about 500, very few of which are not included in one or the other of the lists referred to above. He also points out the absence of the natural orders: Dilleniaceæ, Piperaceæ, Guttiferæ, Ternstroemiaceæ and Gesneraceæ, which, for most of the orders, is certainly remarkable. It is true that Brace and Gardiner's list includes both *Mammea americana* and *Calophyllum*

Calaba (Guttiferæ), but the former is not represented in the Kew Herbarium from the islands, and Brace's specimen of the latter is accompanied by a note to the effect that it was introduced in the Bahamas. Comparatively recent explorations (22 and 23) in the Keys of Florida have yielded some interesting results, to which I will only very briefly allude. Wallace (24), describing the North American flora, says: "When we reach the southern extremity of Florida we still find ourselves in the midst of oaks, sumachs, magnolias, vines and other characteristic forms of the temperate flora; while the birds, insects and land-shells are almost identical with those found farther north. But if we now cross over the narrow strait, about fifty miles wide, which separates Florida from the Bahama Islands, we find ourselves in a totally different country, surrounded by a vegetation which is essentially tropical and generally identical with that of Cuba."

From the facts given above, it follows that Wallace was quite right respecting the sudden cessation of northern types in Florida; but a more thorough exploration of Southern Florida has revealed the existence of a large number of essentially tropical plants, common in the West Indies, and having their northern limit in the extreme South of Florida.

The last of the West Indian Islands to which I have to refer are the Caymans (25), explored by Mr. W. Fawcett. The great Cayman is about 180 miles distant from Jamaica and Cuba, and is only a mere speck of land, seven or eight miles across in its broadest part; yet its vegetation is evidently the remains of a larger flora rather than a derived one. Mahogany, cedar, logwood and fustic, the characteristic trees of the region, form the bulk of the arboreous vegetation. Upwards of 100 species of vascular plants were collected during a short visit, and they included two orchids new to science, namely, *Dendrophylax Fawcettii* and *Schomburgkia Thomsoniana*.

Dr. Urban's proposed general Flora of the West Indies is being preceded by critical elaborations (26) of many of the obscure and difficult genera, especially those numerous

in species which have been variously limited by different authors working at a portion only of them. Many supposed anomalies in distribution disappear when the whole material is handled by one person.

In all my references and comparisons I have purposely omitted the cellular cryptogams; but, for the benefit of those persons interested, I may add here that since 1892 the work under the Committee of the British Association has been almost entirely on these lower organisms; and already several important contributions to the literature have been published, and others are in course of preparation.

BIBLIOGRAPHY.

(1) Report of the Committee . . . appointed for the purpose of reporting on the present state of our knowledge of the Zoology and Botany of the West Indian Islands, and taking steps to investigate ascertained deficiencies in the Fauna and Flora. Read at the Bath Meeting of the British Association, 1888. *Botany*, by W. B. Hemsley; *Zoology*, by D. Sharp.

(2) G. S. JENMAN. In *Bulletin of the Botanical Department of Jamaica*, beginning in No. 18, Aug., 1890.

(3) J. G. BAKER. On the Vascular Cryptogams of the Island of Grenada, in the *Annals of Botany*, vi., pp. 95-102, 1892.

(4) *British Association Committee's Report*, 1891.

(5) Flora of St. Vincent and Adjacent Islets. *Kew Bulletin of Miscellaneous Information*, pp. 231-296, 1893.

(6) H. F. A. EGGERS. The Flora of St. Croix and the Virgin Islands. *Bulletin of the United States National Museum*, No. 13, p. 133, 1879.

(7) A. GRISEBACH. *Gesammelte Abhandlungen*, p. 264.

(8) H. EGGERS. Supplement til St. Croix's og. Jomfruöernes Flora, in *Videnskabelige Meddelelser fra den Nat. Foren.*, i. Kjöbenhavn, pp. 11-21, 1889.

(9) Hooker's *Icones Plantarum*, xv., t. 1401.

(10) A. STAHL. *Estudios sobre la Flora de Puerto Rico*, 1883-1888.

(11) DON DOMINGO BELLO Y ESPINOSA. Apuntes para la Flora de Puerto Rico, in *Anales de la Sociedad Espanola de Historia Natural*, x., pp. 231-304, lam. 4 y 5, xii., pp. 103-130, lam. 1, 1883. The descriptions repeated in Engler's *Jahrbücher*, vi., *Literaturber.*, pp. 58-65, 1885.

(12) H. EGGERS. Reise in das Innere von St. Domingo, in Petermann's *Mittheilungen*, pp. 35-41, t. 3, 1888.

(13) W. B. HEMSLEY. In *Gardeners' Chronicle*, n.s. xvii., p. 263, 1882.
(14) De Candolle's *Prodromus*, xvi., 2, p. 138.
(15) DESCOURTILZ. *Flore des Antilles*, vii., p. 5, t. 453.
(16) L. G. TIPPENHAUER. *Die Insel Haiti*, 4to., pp. 693, illustrated, 1893.
(17) Engler's *Jahrbücher*, xvii., *Literaturber.*, p. 25.
(18) JOHN GARDINER and L. J. K. BRACE. Provisional List of the Plants of the Bahama Islands. Arranged with Notes and Additions by C. S. Dolley, in *Proceedings of the Academy of Natural Sciences of Philadelphia*, pp. 349-407, 1889.
(19) A. S. HITCHCOCK. List of Plants collected in the Bahamas, Jamaica, and Grand Cayman, in the *Fourth Annual Report of the Missouri Botanic Garden*, pp. 47-179, 1893, with four plates. There are also separate copies.
(20) H. F. A. EGGERS. Flora of the Bahamas, in *Nature*, xxxvii., pp. 565, 1888.
(21) J. G. BAKER. *British Association Committee's Report*, 1888.
(22) J. COSMO MELVILL. List of the Phanerogams of Key West, South Florida, in *Memoirs of the Manchester Literary and Philosophical Society*, 3rd series, viii., pp. 138-154, 1884.
(23) J. M. HOLZINGER. List of Plants new to Florida, in *Contributions from the U.S. National Herbarium*, i., p. 228, 1893. The supplement to Chapman's *Flora of the Southern United States*, 1883, it may be added, is largely composed of West Indian species.
(24) *Island Life*, p. 5.
(25) W. FAWCETT. Report on the Cayman Islands, in the *Bulletin of the Botanical Department, Jamaica*, No. 11, 1889.
(26) J. URBAN. Additamenta ad cognotionem Floræ Indiæ Occidentalis, in Engler's *Jahrbücher*, xv., pp. 286-361, 1892-3. Also reprinted with both a separate and the original pagination.

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PEPTONE.

IT was in the early years of the present century that the most important discovery was made that the secretion of the glands of the stomach is an acid one; and Carl Schmidt was the first to show with analytical certainty that it is hydrochloric acid which is *par excellence* the acid of the gastric juice. The presence of other acids like lactic acid is more or less accidental. The absence of putrefaction in the normal gastric contents was noted by Spallanzani, and is caused by this acid. There can be no doubt that the antiseptic action of the juice, which is very great, serves us in good stead by protecting us very largely from the evil results which would otherwise follow the introduction of numerous microbes with every meal. But it is with gastric juice as a digestant that we have now to deal. The first observers were inclined to attribute the solvent power of the juice to its acid, but as Dr. Beaumont showed in his classical observations on Alexis St. Martin which have laid the foundation of all our modern knowledge on digestion, this could not be the case. An acid of the same strength is a less powerful solvent, and therefore the gastric juice must contain a special solvent principle. This Eberle supposed to be the gastric mucus, a supposition easily refuted. It was Schwann who discovered this special principle and called it pepsin. He gave the name albuminose to the product of its action on albumin; Lehmann's name peptone, however, has since been generally adopted. Lehmann recognised that peptone is not coagulated by heat as albumin is.

The modern conception of the process of proteolytic digestion in the stomach is the following: Gastric juice acts on proteids in virtue of the compound between pepsin and the acid which it contains. This compound may be styled pepsin-hydrochloric acid. Like that of most other ferments its action is a hydrating one, and similar products may be obtained by other hydrating agencies, such as

heating with dilute mineral acids or superheated steam. The final product of this action is called peptone, and this substance compared to the original albumin is very diffusible. But between the albumin and the peptone are several intermediate stages of intermediately diffusible substances. One of these produced in small quantities is acid albumin, but the greater number come under the general heading of the proteoses.

The pancreatic digestion of proteids is closely similar; it, however, occurs in an alkaline medium, and is more energetic, and, moreover, if long continued leads to a breaking up of some of the peptone into simpler nitrogenous substances like leucine, tyrosine, aspartic acid, and ammonia.

It should be mentioned that though highly diffusible compounds like proteoses and peptone are formed in the alimentary canal, none find their way as such into the circulating blood, even during the periods of most energetic digestion. These substances are in fact pretty powerful poisons when injected into the blood stream. The epithelial lining of the alimentary canal normally protects us from these toxic products by once more dehydrating the peptone in virtue of the vital activity of its cells. Though lymphoid tissue, which is abundant in the intestinal walls, has been considered by some to have a share in this action, most observers are pretty well agreed that it is the columnar epithelium which is the main agent in the "regeneration of albumin".

But to return to the products of digestion, it was very soon recognised that they are numerous; Meissner described the varieties of peptone as parapeptone, dyspeptone, metapeptone, *a*, *b* and *c* peptone. Schmidt-Mulheim distinguished between parapeptone, propeptone and peptone. Parapeptone is the acid albumin, and propeptone is a very good name for what we now call the proteoses. Nearly all of our present knowledge of the chemistry of digestion is due to the work of Kühne and those associated with him in his researches, particularly Chittenden and Neumeister. A most valuable method of isolating peptone was discovered by Wenz, one of Kühne's pupils. It consists in the use of

ammonium sulphate as a reagent; when added to saturation this salt readily precipitates all proteids except peptone. Pure peptone was never obtained previous to this, but always more or less mixed with proteoses.

The earliest of Kühne's observations showed him that there are two varieties of peptone, *hemi-peptone*, which by the pancreatic juice is further split into leucine, tyrosine, etc., and *anti-peptone*, which resists this action. The corresponding intermediate proteoses may be termed *hemi-albumose* and *anti-albumose* respectively.

More recent observations have shown that albumoses may be classified in another way, according to their reactions and solubilities, into:—

1. Proto-albumose; soluble in hot and cold water and dilute saline solutions, but precipitated by saturation with sodium chloride or magnesium sulphate.

2. Hetero-albumose; insoluble in water, and therefore precipitable by dialysing out the salt from its solutions. Otherwise it closely resembles proto-albumose.

3. Deutero-albumose; soluble in hot and cold water, and not precipitated by saturation with sodium chloride or magnesium sulphate, but it is by saturation with ammonium sulphate. It is thus in its reactions nearest to the peptones.

Peptone itself resembles these albumoses by giving the biuret reaction (pink colour with copper sulphate and caustic potash), but differs from them in not being precipitable by ammonium sulphate nor by nitric acid.

Neumeister has succeeded in discovering the relationship between these two classifications of the albumoses. Albumin may be considered to be composed of hemi-albumin and anti-albumin; the hemi-albumin in the first stage of hydration is split into proto-albumose and hetero-albumose; the anti-albumin yields hetero-albumose and acid albumin. The next stage in hydration is deutero-albumose, and the final step is the conversion of the deutero-albumose into peptone of the hemi and anti varieties.

The albumoses (proto and hetero) formed directly from the albumin may be called primary albumoses.

Deutero-albumose is a secondary albumose, and is thus nearest to the peptones not only in its reactions but also in its order of formation.

The similar products formed in the digestion of globulin may be called globuloses; of vitellin, vitelloses; of casein, caseoses; of myosin, myosinoses; there are individual minor differences, but all are closely similar to the albumoses already described, and the general term proteoses includes them all. The products of digestion of elastin, and of gelatin, have also a general resemblance to the proteoses and peptone.

Such briefly is a summary of our knowledge of proteolysis produced by gastric digestion. But within the last year or two further points of detail have been taken up and worked out, and it is to these that it is the special object of this paper to draw attention. In so doing it will be necessary to restrict our consideration to those papers in which peptones and albumoses are treated from the digestion point of view. It would lead us too far to take up another branch of this subject which has recently attracted so much attention, namely, the chemical action of micro-organisms, and the poisons they produce. Suffice it to say that in numerous instances the toxines, and antitoxines of bacteriologists, are proteoses, or substances closely allied to them.

Pekelharing[1] has attempted to throw doubt upon the individuality of peptone and upon the ammonium sulphate method of isolation, claiming that proteoses are only partially precipitated by the ammonium salt, and that the so-called peptone is merely a mixture of albumose or proteose, with some unknown substance or substances. In support of this view he apparently finds it impossible to prepare a peptone which will not yield some proteose by treatment with ammonium sulphate, or which will not show the presence of proteose by such reagents as trichloracetic acid.

This criticism has led to renewed research on the part

[1] *Centralblatt f. Physiol.*, vii., p. 43.

of Kühne[1] in Heidelberg, and of Chittenden[2] who now works independently of his old master in the laboratory of Yale University.

In his first paper Kühne states in general terms that a solution containing a mixture of proteoses and peptone gives a precipitate of proteoses when saturated with ammonium sulphate, the peptone remaining in solution. After filtration, the filtrate if set aside will subsequently give a further precipitate if more salt is added. This has been explained by supposing that the saturation was in the first case incomplete, or that the peptone is partially changed back into proteose. He proceeds to show that the former is the more probable explanation. There are many precautions necessary in order to precipitate the last traces of proteose. It is necessary in the first instance to use large volumes of the saturated solution in addition to merely adding crystals of the salt to the proteid mixture. Further, it is found that whereas the greater part of the proteose is precipitated by the salt if the reaction of the mixture is made acid, the residue, which is difficult of precipitation, comes down more readily if the reaction is made alkaline. It is further necessary, after the solution of peptone is obtained, to remove the salt employed ; this may be accomplished by the use of barium carbonate after concentration. If pancreatic juice is used for the preparation of peptone, care must be taken to remove leucine and tyrosine also. In drying, concentrating, etc., especially if sulphuric acid is used, a brownish product is formed ; this is minimised by care in the manipulations. This substance is precipitated by ammonium sulphate ; it is not however albumose ; it gives no biuret reaction. Further, if a precipitate forms on dialysis, it is not necessarily of proteid nature ; if hard water is used it may be calcium sulphate.

Pekelharing does not seem to have recognised the necessity of these and other precautions and details ; and, moreover, the differences between peptone and proteose are very striking. This aspect of the subject is considered

[1] *Zeit. Biol.*, xxix., pp. 1 and 308. [2] *Journ. of Physiol.*, xvii., p. 48.

more fully in Kühne's second paper. There are not only differences in solubility, but differences in elementary composition, differences in behaviour to such reagents as metaphosphoric and trichloracetic acids; and peptone when dry stands alone among the products of proteolysis, by hissing like phosphoric anhydide does on the addition of water.

Chittenden takes up the matter from rather a different point of view. He has considered it necessary to repeat his old experiments with as pure a specimen of proteid as he has been able to obtain, instead of using white of egg or other similarly impure raw materials. The proteid he has selected is the crystallised globulin or vitellin from hemp seed. He also entirely disagrees with Pekelharing, as under these more stringent conditions he confirms his older work; he, however, makes out a few new points, and so a brief *resumé* of the entire paper may not be unprofitable.

He performed two separate digestions on a large scale, one of which was continued for three, the other for ten days. The products of digestion were carefully separated from one another and analysed; their reactions are exhaustively described, including their specific rotatory power. In separating proteoses from peptone the difficulties of manipulation are recognised, and so far as is at present possible obviated. The investigation, however, lends no support to Pekelharing's views, but is in complete accordance with Kühne's contention that peptone is a definite, well-characterised substance. Its amount increases, and that of proteose diminishes as digestion progresses, but an artificial digestion in which all proteose is converted into peptone has not yet been obtained. The progressive changes from primary proteose to deutero-proteose and peptone are most marked in the early stages of digestion; the disappearance of deutero-proteose and the formation of peptone is subsequently a very gradual process.

In the proteolytic process, at least four products of hydration and cleavage are easily recognisable, namely, proto-proteose, hetero-proteose, deutero-proteose and peptone. The examination of the percentage composition of

these substances shows a gradual and progressive falling off of carbon as digestion progresses. But the tables given show another very noticeable feature, and that is that the percentage composition of the individual proteoses differs in the two digestions; the substance called proto-vitellose, for instance, in the digestion carried on for three days, cannot be the same as that with the same name in the digestion carried on for ten days, although both give the same reactions, specific rotation of the plane of polarised light alone excluded. This cannot be due to analytical errors; a skilled investigator of Professor Chittenden's eminence could not fall into such a mistake; besides other proteids, egg-white, for instance, under the influence of superheated steam gives similar results.

The explanation advanced is that the four substances named merely represent the main steps in the hydration process, but that there are further links in the chain mixed with these, which cannot at present be separated, and which manifest the same general chemical behaviour.

Such a result is not altogether unexpected; it only shows how, in spite of the advance of chemistry as a whole, it is practically at a standstill so far as the proteids are concerned; nor can we hope to understand the steps in proteolysis until the chemists are able to give us some idea of the chemical constitution of the proteids which these digestive juices act upon.

One of the properties alluded to, which the proteoses and peptones agree in possessing, is their power of diffusing through membranes; and although these products of digestion do not avail themselves of this to the full, for we never find them in the blood, it can hardly be doubted that the formation of diffusible from indiffusible substances must fulfil some useful end. It appears to be the main object of all digestion, whether of proteids or carbohydrates. It may be held that it enables the substance to be absorbed to start on its journey, though in the progress of that journey bloodwards the property in question is lost.

The same two names are associated with the investigation of this point with accuracy. They worked indepen-

dently and arrived at the same result. To take Chittenden[1] first. He found that the proteoses though diffusible were not nearly so much so as peptone. A curious fact which was unexpected was that deutero-proteose is less diffusible than proto-proteose. Proto-gelatose is fairly diffusible, but it has a somewhat lower endosmotic equivalent than the corresponding proteose. Elevation of temperature increases the rate of osmosis, especially of the proteoses.

I give in conclusion the figures from Kühne's paper,[2] which illustrate these facts. Hetero-proteose is the least diffusible of the proteoses; in neutral saline solutions it is precipitated, and none passes through the dialyser; dissolved in ammonia it loses 5·22 per cent. Deutero-proteose comes next (loss 24·1 per cent.); then proto-proteose (loss 28·3 per cent.); while peptone loses 51 to 51·8 per cent.

W. D. Halliburton.

[1] *Journal of Physiol.*, xiv., 483. [2] *Zeit. Biol.*, xxix., 1.

BUDDING IN TUNICATA.

THE differences between embryogenesis and blastogenesis have often been insisted upon, and in no group more emphatically than in that of the Tunicata. The same or homologous organs have been shown in some cases, and stated in others, to arise in the larva from ectoderm, in the bud from endoderm. It has accordingly been asserted by various writers that the phenomena of budding are at variance with the axioms of the germ-layer theory, and seriously impugn the validity of current ideas upon the differentiation of the germinal layers. This attitude seems to me to be at all events premature. The asserted irregularities of bud development have been corrected in more than one important instance; and, with one exception, the organs whose origin in the bud has been regarded as most inconsistent with the germ-layer theory are precisely those organs concerning the facts of whose development there is most dispute. Difficulties and doubtful points are plentiful enough; but, with regard to the former, I shall endeavour to show in the present article that they have been exaggerated and are not insuperable; and, so far as the latter are concerned, it will naturally be necessary for us to await the results of renewed investigations before we can arrive at any decisive conclusions upon them. I shall be surprised if further research does not continue to remove the apparent discrepancies between bud and larval development, although it is far from my intention to prejudge the important points at issue. It is rather my desire by attempting a critical summary of our existing knowledge to facilitate to some extent the solution of the debated points.

1. *The Participation of the Germ-layers.*—In diploblastic animals budding is reduced to its simplest terms. In the calcareous sponges, for example, every stage can be traced between mere sacculation of the body wall—involving outgrowths of both germ-layers—and the complete formation

of osculum-bearing persons. No simpler type of budding can be conceived than that exhibited by the Asconidæ. In the Hydrozoa the phenomena are practically identical. An attempt was made, it is true, by Albert Lang (18), one of Weismann's pupils, to derive the buds of Hydroidea from the ectoderm alone ; but the recent researches of Seeliger (28) have fully confirmed our earlier ideas upon the subject. Budding in Hydrozoa involves the participation of both germ-layers. The buds of the Diploblastica are two-layered.

It is not an object of the present article to make a general survey of budding in the Triploblastica. So far as the Tunicata are concerned, no fact has been more clearly established than this,—that in every one of the different types of budding found within the group the two primary germ-layers of the parent directly participate ; the ectoderm and endoderm of the buds are direct prolongations of the corresponding layers of the parent. The only instance that is likely to be cited as an exception to this statement is that of the Botryllidæ, where the inner layer of the bud is a prolongation of the atrial epithelium of the parent. Hjort (9, 10) maintains that the inner layer is consequently of ectodermal origin, a point which, if true, would render utterly useless any attempt to reconcile the phenomena of blastogenesis in Tunicata with the theory of differentiated germinal layers, since the whole of the pharynx and digestive tract of the bud is derived from the internal layer. Hjort's conclusion, however, is founded entirely on the analogy of those Ascidians in which the peribranchial chambers have been shown to be derived from ectodermal involutions ; whereas it has been shown by Pizon (20) that in the embryos of Botryllidæ the peribranchial chambers are exclusively derived from endodermal diverticula. It is the old story of the subtlety of nature exceeding the subtlety of human wit.

But the buds of Tunicata are not built up exclusively from the two primary layers. In all cases which have been properly examined some derivatives of the mesoblastic tissues of the parent are also included within the bud,

between the endodermal vesicle and the ectodermal envelope. These derivatives generally take the form of wandering mesenchyme cells, and are swept along between the two primary layers by the force of the maternal blood current. Many of them become applied to the epithelial walls of the developing bud, and build up its musculature and other skeletotrophic tissues.

The Tunicate bud in its typical form may accordingly be said to consist of two epithelial vesicles one inside the other, and of a certain quantity of mesenchymatous tissue between the vesicles. The two vesicles are derived by evagination from the two primary layers of the parent, and are at first in direct continuity with them; the mesenchyme is also derived from the corresponding tissue of the parent. Even in the minute ventral buds of *Distaplia* Salensky (25) has recently shown that several parental mesenchyme cells are included which furnish the rudiment of the future mesodermal tissues. On the other hand the statements of Korotneff (12) upon the triploblastic nature of the buds of *Dolchinia* must be accepted very cautiously; for not only are we without any actual evidence as to the origin of the three elements he describes, but their later fate renders the nature which Korotneff has ascribed to them extremely doubtful.

So far as the evidence goes, therefore, the Tunicate bud is composed of derivatives of the three germ-layers; and in this group of the Triploblastica at any rate the process of blastogenesis is in complete harmony with the germ-layer theory so far as the constitution of the buds is concerned.

Nevertheless, although I am not aware that any investigator has satisfactorily established any instance of a Tunicate bud possessing an organisation simpler than this, it is an interesting fact that in certain cases the bud exhibits a greater complexity. In the four primary ascidiozooids of *Pyrosoma*, for example, not only are the general ectoderm, endoderm, and mesoderm of the parent cyathozooid prolonged to form the corresponding tissues of the buds, but a pair of special prolongations of the peribranchial sacs is also contained in the developing stolon. Instead of being triploblastic, the buds become actually four-layered in this

case, owing to the addition of another element or organ-process to the three layers typically present. In *Salpa* also the buds are four-layered from the beginning. The peribranchial sacs of the stolon, unlike those of the primary ascidiozooids of *Pyrosoma*, develop quite independently of the parental organ; but a genital rod derived from the parent is present throughout the stolon and supplies a fourth element to each of the buds. It is true that this particular element takes little or no part in the formation of the somatic tissues of the bud; but, as the presence of generative organs is a characteristic feature of the asexual generation of Salps, it is important to observe that these organs are not secondarily developed from one of the primary layers of the bud but are directly derived from the preceding generation. This transference of reproductive elements from one generation, either sexual or asexual, to another, which is always produced by budding, is a striking feature of Tunicate blastogenesis. It has been thoroughly established in several forms, *e.g.*, *Salpa* (Brooks), the secondary ascidiozooids of *Pyrosoma* (Joliet, Seeliger), *Botryllus* (Pizon); and it possibly occurs also in other forms such as *Clavelina* and *Perophora*, although the similarity between the young germ-cells and the general mesenchyme of these types has hitherto prevented any exact demonstration of the point.

The constitution of the bud would appear to be still more complicated in the genus *Doliolum*. Both Grobben (8B) and Uljanin (31) state that in this form the ectoderm envelope encloses as many as five separate kinds of embryonic elements, of which some are unpaired and some paired. The origin and fate of these constituents, however, is still so imperfectly ascertained that it seems to me to be altogether impossible to make any detailed use of the current statements upon the budding of *Doliolum*. It seems to be fairly well established, however, that the stolon (and buds) of *Doliolum* contain two pairs of prolongations from the pharynx and cloaca of the parent, and an unpaired outgrowth of the parental mesoderm. The paired outgrowths of the pharynx I regard as homologous with the epicardial tubes of Ascidians, and the diverticula from the cloaca readily lend

themselves for comparison with the paired cloacal or peribranchial canals of the primary ascidiozooids of *Pyrosoma.* Uljanin's statements that the pharyngeal outgrowths give rise partly to the genital glands, and that the cloacal outgrowths furnish the rudiments of the musculature and nervous system of the bud, are, however, most improbable. There is no inherent difficulty in the origin of the nervous system from the cloacal outgrowths, since the cloaca of *Doliolum* is an ectodermal structure; but it is impossible to believe, upon the strength of Uljanin's optical sections alone, that a special pair of cloacal prolongations gives rise to the musculature as well as the nervous system, especially as both Grobben and Uljanin assure us that the stolon actually contains a special mesodermal rudiment. Both authors trace the development of the pericardium from this element, and Grobben derives the general musculature from it also. It is pardonable, therefore, if we refrain for the present from assenting to Uljanin's discordant conclusions upon the fate of the cloacal outgrowths. We cannot in this case apply the same general considerations in support of a common origin of nerve and muscle from the ectoderm that were originally urged in favour of Kleinenberg's contention in the case of *Lopadorhynchus.* Kleinenberg's views, I believe, have failed to be confirmed, reasonable as was the position of their author; but the particular mode of origin described by Uljanin in *Doliolum* is altogether unprecedented and circuitous. Recent research, moreover, has shown conclusively in the case of *Salpa* (Brooks and Korotneff), *Pyrosoma* and *Distaplia* (Salensky) that the neural rod in the stolons of these forms is a local differentiation of the ectoderm at the base of the stolon; and I am, therefore, strongly inclined to believe that the neural rudiment in the *stolo prolifer* of *Doliolum* will also eventually be traced to an early proliferation of the thickened ectoderm which marks the site of the commencing stolon.[1] Criticism thus drives us back from Uljanin's conclusions to the position previously occupied by Grobben as to the nature of the

[1] Grobben, taf. iv., fig. 22; Uljanin, taf. viii., figs. 4 and 5.

cloacal diverticula. They may be regarded, at any rate provisionally, as the forecasts of the cloacal chamber of the future zooid,—a view which would justify the comparison I have drawn above between them and the otherwise unique peribranchial canals of the *Pyrosoma* stolon.

It is also very difficult to accept Uljanin's account of the fate of the pair of pharyngeal outgrowths. According to this investigator each of these elements soon divides, and one of the portions unites with its counterpart to form the unpaired rudiment of the pharynx and alimentary canal of the bud, while the remaining parts constitute the paired forecasts of the genital gland. Analogy is altogether against such a state of things as this. In no other Tunicate do the pharyngeal outgrowths (=epicardial tubes) give rise to the generative glands. These invariably arise either from the mesoderm, after its differentiation from the endoderm, or directly from the generative gland of the parent. Grobben's statement that the alimentary canal and generative organs of the bud arise from parental elements which are distinct from the first is much more likely to be true, since it has the force of analogy in support of it.

Be this as it may, it is quite clear that in the formation of buds in the Tunicata all three germ-layers of the parent participate. In the simplest cases the bud is composed of the three undifferentiated layers alone (*e.g.*, *Clavelina*, *Distaplia*, *Amarœcium*, *Perophora*); but in the majority of instances the bud consists in its earliest stages of parental material which has already undergone differentiation to a greater or less degree. In these cases the bud consists of an aggregation of a number of organ-rudiments instead of the three undifferentiated layers; and in all satisfactorily investigated cases the special elements of which the bud is composed are direct prolongations or derivatives of the corresponding organs of the parent. The nervous system seems rarely to be prolonged into the bud in this manner, but to be derived anew by delamination. *Diplosoma*, however, furnishes an exception to this rule, as the recent researches of Salensky (27) show; and Pizon has endeavoured to make out the same phenomenon in the

case of Botryllus (20). The other organs or tissues of the bud which may be produced directly from the parental organs are—1. The peribranchial chambers, *e.g.*, primary ascidiozooids of *Pyrosoma*, *Doliolum* (?); 2. the pericardium, *e.g.*, *Pyrosoma*, *Salpa*. (In *Doliolum* the pericardium of the larva and the pericardial rudiment of the stolon are separated off from the mesoblastic bands at the same time and in juxtaposition.) 3. The genital glands, *e.g.*, *Pyrosoma*, *Salpa*, *Doliolum* (?), *Botryllus*.

The two kinds of budding which have just been described are sufficiently distinct to be entitled to receive appropriate names, and the nature of the distinction would, I think, be adequately indicated by the use of the terms "differentiate" and "undifferentiate" in connection with them. By "differentiate budding" I mean the production of buds by means of a number of special prolongations of the differentiated parental organs; and by "undifferentiate budding" the production of buds by means of the three undifferentiated germinal layers alone. *Botryllus* furnishes a connecting link between the two types. In this form the first few generations of buds are produced upon the undifferentiate plan, but in later generations the participating mesoderm is differentiated to some extent—the genital cells no longer arise from the mesoderm proper to the bud, but, as Pizon has shown, are transferred in a more or less matured condition from one generation to another. In *Clavelina*, also, the mesodermal organs of the bud are derived from two distinct sources—the musculature, connective tissue, and blood from the mesenchyme of the parent, the pericardium and heart, on the other hand, from the endodermal (epicardial) prolongation. In spite of the absence of a sharp demarcation between the two modes of budding, I think it desirable to emphasise the contrast between the more typical cases; and it is a most noteworthy fact that while the "undifferentiate" type is especially characteristic of the fixed Ascidians (Ascidiacea), the "differentiate" type is peculiar to the pelagic forms (Thaliacea). *Botryllus*, on account of the continuity of its germinal cells through successive generations, furnishes an interesting instance

among the fixed Ascidians of a phenomenon of blastogenesis which reaches its most striking development in the Thaliacea (especially *Pyrosoma* and *Salpa*).

At the present moment I do not wish to prejudice the question as to which of these types of budding is the more primitive. One is always inclined to regard the simpler of any two phenomena of the same kind as the more primitive, and on this account the "undifferentiate" type, characteristic of the Ascidiacea, at first sight naturally appears to be the primitive form of asexual reproduction, of which the "differentiate" type is a later and secondary elaboration. The question cannot be adequately discussed, however, before a fuller survey of the phenomena has been made; and the treatment of it will therefore be postponed to a later section of this article.

2. *The Origin of Special Organs in the Bud.*—Remarks have been made in the preceding section which indicate that the same or homologous organs may develop in the buds of different types by processes which are not always identical, and that these may also be dissimilar from the processes typical of embryonic development. The literature upon the subject is full of such instances; and even if only half of these asserted instances are actually true, development by budding must be regarded as capricious and irregular to an extraordinary degree. The writer who endeavours to trace the order underlying a mass of conflicting statements about phenomena which in themselves are remarkably varied, has to avoid a double danger,—the risk of wasting his efforts by an excess of timidity in dealing with the materials before him, and the tendency to constrain the facts into a system more rigid and narrow than nature has prescribed. In the present case I scarcely hope to be successful in avoiding both these dangers; but it certainly seems most desirable that some attempt should be made to estimate the value of the conflicting statements which exist, and to systematise the facts which appear to have been most satisfactorily ascertained.

The development of the peribranchial sacs has perhaps furnished the chief difficulty in attempts to apply the germ-

layer theory to the process of blastogenesis in Tunicata. The ectodermal origin of the peribranchial involutions in *Pyrosoma* and many Ascidians, and the analogy of *Amphioxus*, have induced many authors to regard these organs as universally ectodermal. On the other hand no fact has been more frequently demonstrated than the endodermal origin of the peribranchial chambers in the Ascidian bud. The inconsistency of this fact with the above-mentioned view of the ectodermal nature of the organs has accordingly led many writers to give up any attempt to reconcile the phenomena of budding with the germ-layer theory which has sprung out of modern embryological research. There is, however, a less desperate way out of the difficulty before us. It is possible that the peribranchial sacs are not entirely ectodermal by nature: there still remains the view, propounded in the first instance, I believe, by Fol (7), that the peribranchial sacs of Ascidians correspond to the branchial canals of Appendicularians, and, therefore, to a pair of gill-slits formed by the union of a pair of ectodermal involutions with a pair of endodermal outgrowths. The nature of the peribranchial sacs, according to this view, is neither purely ectodermal nor purely endodermal, but a mixture of both. Van Beneden and Julin (3) endeavoured to establish this view as a result of their classical researches upon the development of *Clavelina;* in embryos of this form the peribranchial sacs seemed to them to be developed from both germ-layers. Their statements upon this point, however, have recently been controverted both by Willey (32) and Seeliger (30), who maintain that the peribranchial sacs are formed from ectodermal involutions alone. Yet the mistake must not be made on this account of imagining that Fol's suggestion as to the primitively double origin of the peribranchial sacs derives no further support from embryology. In *Salpa*, according to the recent work of Korotneff (14) and the older researches of Salensky (23), the cloacal cavity is directly derived from that of the pharynx; and since its walls are stated to be formed from the same layer as those of the pharynx (the endodermal histogenes of Korotneff), there seems to be some evidence that in this form at any rate the cloacal and peribranchial

cavities are not of ectodermal origin. It would be unwise, however, to attach too much weight at present to inferences drawn from the embryology of *Salpa*, since extensive secondary changes render the interpretation of its development a work of unusual difficulty. But no objection of this kind can be urged with regard to the embryology of *Botryllus*. In this peculiar, and, as I think, primitive type of Ascidians, Pizon (20) showed a couple of years ago that the cloaca and peribranchial chambers result entirely from the transformation of a pair of diverticula from the endodermal vesicle (or primitive pharynx) of the larva. The only ectodermal element is provided by the lips of the cloacal aperture. Pizon's observations have not hitherto been confirmed, but from the researches on this interesting form upon which I am myself engaged, I am inclined to attach full value to his principal results. It certainly seems unjustifiable on the part of Hjort (10) to decline to believe Pizon's statements on the sole ground that in *Phallusia* and *Clavelina* the development of the peribranchial chambers takes place differently.

Thus, apart from general considerations, there is some direct evidence that the peribranchial sacs in Tunicate embryos are occasionally endodermal in origin; and this lends corresponding support to the views which Fol, Van Beneden and Julin have expressed as to the homologies and nature of these structures. It is true that there is no clearly established case, either among buds or larvæ, in which the peribranchial sacs have been shown to arise from both elements; but a little consideration will show that this is no insuperable objection to the theory of their primitively double origin. For when the primitive pair of gill-slits lost their cilia (as, for example, has undoubtedly occurred in the Appendicularian *Kowalevskia*), and dilated to assume the functions of mere channels for the currents of water passing through the newly-formed pharyngeal stigmata, there is every reason to expect that the circuitous mode of development—by the participation of both layers—which was inherited in their earlier phases, would be replaced by a more direct and simpler process. One of the two elements

would gradually predominate to the exclusion of the other, so that the entire water-channel on each side would eventually develop from one instead of both of the layers. There is no apparent reason why the predominating element should in all cases have been the ectoderm; and I regard *Botryllus*, and possibly *Salpa* too, as one of the forms in which the endodermal portion of the primitive gill-slit has enlarged to form the whole of the peribranchial sac.

It will be admitted that if the theory which I have here set forth upon the phylogeny of the cloaca and peribranchial sacs is true, the variable origin of these structures in the buds of Tunicata ceases to offer any serious difficulty. In the buds of all fixed Ascidians so far investigated these organs arise as outgrowths from the endoderm; while in *Pyrosoma*, *Anchinia*, and *Dolchinia* they undoubtedly arise from the ectoderm. If the theory in question be admitted, not only does this diversity of origin cease to be inconsistent with the doctrine of germinal layers, but it actually furnishes an additional and striking argument in its support.

With regard to the development of the stigmata in the bud, I have elsewhere shown that it represents a simple abbreviation of the phylogenetic mode of origin, which is recapitulated in the embryos of certain types (8).

The endodermal origin of the pericardium and heart in the buds of certain Tunicata (*e.g.*, *Clavelina*, *Circinalium*, *Botryllus* (?), *Anchinia*, *Dolchinia*) can scarcely be regarded as a very serious difficulty. Not only is a similar origin ascribed to these organs in the embryos of *Clavelina* (Seeliger, Van Beneden and Julin); but, as the mesoderm itself is derived from the endoderm, the independent origin of the heart from the same layer can be readily interpreted as an instance of precocious segregation. It is desirable, however, that future investigators should examine this point with particular caution. In the buds of *Botryllus* the development of the heart has been described independently by Hjort, Oka, and Pizon, and there is a considerable difference between their accounts. Pizon (20) states that the pericardial vesicle is derived as a diverticulum from the

primitive endodermal vesicle; Oka (19), from the junction between the pharynx and left peribranchial sac; whereas Hjort (9) maintains that the pericardial rudiment is not an outgrowth of the inner layer at all. It is possible in this case, as Salensky suggests, that Pizon has not really seen the earliest stage of the pericardium. Salensky's observations on the buds of *Distaplia* (25), together with those of Hjort on *Botryllus*, tend to show that the pericardium, in some cases at any rate, may arise by the grouping of mesoderm cells without any connection with the endoderm; though it is impossible to make a positive statement with regard to either of the above instances. On the other hand, the mesoblastic origin of the pericardium and heart is perfectly clear in the buds of *Pyrosoma*, *Salpa*, and *Doliolum*. In the two first cases the constituent cells are derived directly from the pericardium of the parent; in *Doliolum* from the mesodermal element of the stolon, which is nipped off from the mesoblastic bands of the larva.

The musculature of Tunicate buds seems to be derived from the mesoblast cells lying between the two primary layers—if we except Uljanin's statements with regard to *Doliolum*, which have been criticised above. Pizon's statements concerning the existence of a special band of ectoderm cells which give rise by proliferation to the wandering mesoblast cells in *Botryllus* are entirely without parallel, so that it is impossible to accept them until further evidence is forthcoming. It must be remembered, moreover, that this proliferation of the ectoderm has also been observed by Oka, who states that the cells so derived reunite beneath the dorsal tube to form the dorsal nerve-cord. This assertion, although opposed by Pizon in an appendix to his paper on *Botryllus*, appears to be still worthy of consideration.

The origin of the nervous system in the bud must next engage our attention. Kowalevsky (16) and other earlier investigators derived the nervous system from the roof of the endodermal vesicle, as, for example, in *Distaplia stylifera* (=*Didemnum styliferum* of Kowalevsky), and *Amaræcium*. Seeliger, on the other hand, in the case of

the secondary ascidiozooids of *Pyrosoma* (29), derives the nervous system from the mesoblast; and attributes a common origin to the nervous system and genital cells. Such an origin, if true, would be extremely remarkable; it may be compared with the recent observations of Korotneff, who states (12) that in the buds of *Dolchinia* also the nervous system and genital cells are derived from a common rudiment, although in this case the common rudiment is imagined to be endodermal. It must be borne in mind, however, that the nature which Korotneff attributes to the three primary elements which he describes in the buds of *Dolchinia* is purely hypothetical. And, so far as Seeliger's statements are concerned, it is in the highest degree probable that the ectodermal origin of the nervous system which has been recently demonstrated in the case of *Salpa* by Brooks (4) and Korotneff (14), and in *Distaplia* and the primary ascidiozooids of *Pyrosoma* by Salensky (24, 25), will eventually be extended to the secondary buds of *Pyrosoma*. It is also very probable that Kowalevsky's account of the development of the nervous system in *Distaplia stylifera* is not altogether accurate. In the ventral buds of a species of the same genus Salensky has recently shown that the nervous system develops from a pair of large ectoderm cells, which are differentiated very early in the formation of the stolon, and then sink beneath the general ectoderm. If, as is probable, a similar mode of development also occurs in the posterior buds, it is doubtful whether the methods employed by Kowalevsky would have enabled him to recognise it; and I am inclined to think that he has mistaken for the dorsal nerve-cord an associated organ which will be referred to below as the dorsal tube, and which is undoubtedly of endodermal origin. The observations of Van Beneden and Julin upon the ectodermal origin of the nervous system in the buds of *Clavelina*, a form which is nearly allied to *Distaplia*, support this idea; but, as pointed out by Pizon, the stages described by these investigators were not sufficiently complete to justify us in accepting their results upon this point until further evidence has been obtained.

We now come to *Botryllus*, an exceedingly interesting

form, about which is being waged at present a controversy which must be crucial in its results. The question turns upon the relation between the neural tube and the so-called hypophysis or subneural gland. The existence of this organ, which lies beneath the brain in most Ascidians, has long been known; but its significance has been the subject of much dispute. It has, however, usually been regarded as a diverticulum of the anterior part of the pharynx, homologous with the hypophysis of Vertebrata. Pizon has shown that in the larvæ as well as in the buds of *Botryllus* this organ arises as a forwardly directed tubular diverticulum from the dorsal side of the hinder part of the primitive endodermal vesicle; and that after effecting a secondary union with the wall of the pharynx anteriorly, it loses its primary communication with the endoderm, and gradually atrophies from behind forward until only its anterior portion is left. This persisting portion becomes the subneural gland and ciliated vesicle of the adult Ascidian. To the primary diverticulum, in its whole extent, Pizon has given the descriptive name "dorsal tube". In the bud, Pizon shows that the cloaca and peribranchial sacs are not formed independently of one another, but are constricted off from the dorsal side of the primitive endodermal vesicle as a single saddle-shaped vesicle. It follows from the posterior situation of the diverticulum, which gives rise to the dorsal tube, that the latter also becomes removed from the pharyngeal vesicle *pari passu* with the separation of the cloacal vesicle. In this way the dorsal tube presents itself in later stages of development as a diverticulum of the anterior wall of the cloaca. Pizon's observations upon all these points in the budding of *Botryllus* were fully confirmed by the independent researches of Hjort, whose general conclusions, however, are marred by his assumption of the ectodermal origin of the inner (or endodermal) vesicle. Hjort's attitude with regard to this point has already been discussed. When we pass from the development of the dorsal tube to consider the origin of the nervous system itself, we discover serious differences between the accounts of the two investigators. Hjort maintains that the brain of the *Botryllus* bud is produced by local differen-

tiation of the ventral wall of the anterior part of the dorsal tube; that is, that the brain is of endodermal origin,—if our interpretation of the layers of the *Botryllus* bud is correct. Pizon, on the other hand, is equally sure that the brain and dorsal nerve cord have an origin altogether distinct from the dorsal endodermal tube. According to him the nerve tube is recognisable as a slender thread of considerable length in the stages in which the dorsal tube has acquired its anterior opening into the pharynx, and has not yet lost its primitive posterior communication, and in which the saddle-shaped cloacal and peribranchial vesicle is separated from the pharyngeal vesicle. Anteriorly the nerve-cord lies close beneath the dorsal tube; but posteriorly the two are separated from each other by the cloacal sac. The dorsal tube terminates almost immediately, but the nerve-cord is continued for some little distance backwards between the gut and the floor of the cloaca, until on account of its great delicacy it is no longer recognisable in transverse sections. If Pizon's observations are correct, the nerve-cord is distinct both from the dorsal tube and from all the other organs and layers of the bud from the first, and its juxtaposition to the dorsal tube is to be regarded as of quite secondary importance. Unable to trace any intimate connection between the nerve-cord in its earliest stages and the other organs of the bud, Pizon inferred that the cord is not formed *in situ*, but as a special prolongation from one of the nerves of the parent —an inference which he drew from his observation that the cord in early stages is prolonged backwards beyond the dorsal tube, becoming finer and finer in its course. Possibly in still earlier stages this fine portion of the cord is directly prolonged through the stalk of the bud into the body of the parent; but, as we have seen, the evidence presented by Pizon upon this point is inconclusive. There is still another account of the development of the brain in the buds of *Botryllus*. Oka has also recognised the existence of the dorsal tube in the buds of this form, although he overlooked the earliest stages in its development. He noticed, too, the longitudinal tract of proliferating ectoderm cells. These were regarded by Pizon as a band of incipient mesenchyme

cells; but Oka states that the cells produced along this line of proliferation migrate inwards on each side of the dorsal tube, and reunite on its ventral side to form the brain and nervous system.

There are thus three distinct views as to the origin of the nervous system in the buds of *Botryllus*. According to one the brain is produced from the dorsal (endodermal) tube; according to another from a proliferating streak of ectoderm; while, according to the third, the nervous system is not produced from either of the constituent layers of the bud, but by means of a special rudiment derived from the parent organism. The first view is manifestly improbable, although based on an apparently careful piece of research; the second view finds a parallel in the observations of Van Beneden and Julin on the buds of *Clavelina*, and is quite consistent with the general principles of embryology; the third view is fairly well founded, and equally harmonious with the germ-layer theory. Research alone can settle a point so much disputed; but we may mention that although at first sight the second of these views may commend itself most readily upon *a priori* grounds, the facts upon which this view is based were fully known to Pizon, who entertained the same opinion for a time, and rejected it after renewed investigation. The balance of probability accordingly rests with Pizon's conclusions; and it seems to me that the recent research of Salensky (27) upon the development of the twin-embryo of *Diplosoma* supplies a powerful argument from analogy in support of Pizon's special theory. In this remarkable embryo the primary individual early gives rise (by a process which is intermediate between fission and budding) to a second individual resembling itself; and the nervous system of the second or ventral individual is formed from a special nerve-tube which grows out laterally from the neural tube of the primary individual. Whatever opinion may be held as to the relations between fission and budding in Tunicata the case of *Diplosoma* furnishes a basis of fact which renders Pizon's view of the origin of the nervous system in the *Botryllus* bud worthy of full consideration. It may be that future investigators will

reveal in the buds of *Botryllus* some mode of origin of the nervous system intermediate between that described by Salensky in the case of *Diplosoma* and the merely local differentiation of the ectoderm of the stolon, which has been observed in *Distaplia*, *Salpa* and *Pyrosoma*.

The controversy upon the development of the nervous system in the buds of *Botryllus* raises such wide and important issues that I have thought it worthy of somewhat lengthy consideration. It should also be mentioned that Hjort (10) has recently reiterated the statements contained in his first paper on *Botryllus*, and gives an additional case (*Glossophorum*) in which, as he maintains, the brain arises from the dorsal (endodermal) tube. A similar origin of the brain has just been described by Ritter (21) in the case of the buds of *Perophora*.

Doubtful, however, as many points remain, it has, I hope, been clearly shown in the present article that the theory of differentiated germinal layers has not been proved inapplicable to the phenomena of blastogenesis in the Tunicata. Many of the earlier statements upon the subject were undoubtedly inconsistent with the general theory, but they have been modified to a great extent by the more exact researches of recent years. Development by budding, although presenting extensive peculiarities in the details of organogeny, seems on the whole to agree with embryonic development in all fundamental points. No single fact, inconsistent with the general applicability of the germ-layer theory of development, has been hitherto established in the case of Tunicate buds.

3. *The Types of Budding.*—A classification of the various modes of budding may be made from different points of view. Usually the existence or absence of a stolon is made the basis of classification; but such a course seems quite arbitrary, and raises into fundamental importance a feature altogether secondary and unimportant from a morphological point of view. Some authors are inclined to connect the two processes of budding exhibited by the cyathozooid of *Pyrosoma* and by the Polyclinidæ, on the ground that these

are examples of transverse fission as distinct from budding. But the former is simply an instance of precocious budding, as Korschelt and Heider (15) have shown. And, in the case of the Polyclinidæ, the post-abdomen, although it contains the heart and genital glands, seems—as Giard and Lahille have remarked—to be merely a portion of the stolon into which these organs have been secondarily prolonged. The so-called transverse fission of the Polyclinidæ is therefore only a special mode of stolonial budding, and may be compared with the allied processes exhibited by such forms as *Clavelina* and *Distaplia*. In the former genus the buds remain structurally connected with the parent throughout life; but in *Distaplia*, a form which closely resembles *Clavelina* in structure, the buds produced by the stolon are detached early, and move to the surface of the colony in just the same way as do the post-abdominal segments in Amarœcium and other Polyclinidæ.

In an earlier part of this paper I have drawn attention to the fact that budding in Tunicata takes place upon one or other of two plans, which were termed "differentiate" and "undifferentiate"; and that the former type is especially characteristic of the pelagic forms (Thaliacea), while the latter type is predominant in the fixed forms (Ascidiacea). The distinction between these two types is not, however, sharply marked; and the terms have been suggested for descriptive rather than for classificatory purposes.

A comprehensive survey of the phenomena has led me to the conclusion that the true grounds of classification must be sought in the various modes by which the endodermal elements of the buds originate. It is now well known that in fixed Ascidians the endoderm of the bud is derived in most cases from a pair of diverticula from the posterior region of the pharynx of the parent—the epicardial tubes of Van Beneden and Julin. These peculiar organs are without any homologues in Appendicularians or the higher Chordata, and have clearly been developed within the group in relation with the evolution of budding. Their occasional connection with the heart (*e.g.*, *Clavelina*) must be regarded as a phenomenon of secondary importance. In many Ascidians

the free ends of the epicardial diverticula unite with one another to form an unpaired tube, the cavity of which becomes practically obliterated by the juxtaposition of its walls. This unpaired tube then penetrates the stolon, and usually presents the appearance of a thin broad band dividing the cavity of the stolon longitudinally into two separate blood-channels; under this form it has long been known as the stolonial septum, but its true nature and origin were first elucidated by Van Beneden and Julin. The fusion of the epicardial tubes in *Clavelina* and the majority of the fixed Ascidians should not, however, disguise the fact of their essentially paired nature. In *Doliolum*, as I have shown above, this primitive condition is retained; and in *Distaplia*, as Salensky's recent investigation has revealed, they are still distinct from one another at the time when the first (or ventral) bud is produced. In *Botryllus* also the homologues of the epicardial outgrowths are paired throughout life. In this form, however, they communicate with the pharynx only in their earliest stages, while the peribranchial diverticula are still in open communication with the pharynx in the same region. At a later stage the peribranchial sac and epicardium of each side become separated from the pharynx together; and in the full-grown organism the epicardial tube is a capacious diverticulum of the peribranchial sac. In spite, therefore, of the final difference of position between the epicardial (or perivisceral) sacs of the Botryllidæ and the epicardial tubes of *Distaplia* or *Clavelina*, there can be no doubt, as Pizon has maintained, that there is an exact homology between the two structures. In the buds of *Distaplia*, according to Salensky, the epicardial sacs show a similar connection with the peribranchial diverticula in the earliest stages of their formation; but, at a later period, the peribranchial sacs separate completely from the pharynx, while the epicardial diverticula retain their connection with it.

It appears also, from a recent paper by Ritter (21), that *Goodsiria*, one of the Polystyelidæ, presents the same relations as *Botryllus*.

The primitively paired form of the epicardial diverticula

is therefore retained throughout the life of the parent in *Doliolum*, the Botryllidæ, and the Polystyelidæ. In the two last families buds are produced on each side of the body of the parent, a fact which indicates that the epicardial diverticula were originally independent in function: each provided the endoderm for the bud on the corresponding side of the parent's body. The same conclusion may be drawn from the formation of the single ventral bud of *Distaplia*, since in this case only one of the two epicardial diverticula which are present gives rise to the endoderm of the bud.

In most cases, however, lateral budding has been replaced by ventral budding, and each of the two epicardial outgrowths, which were originally independent both in position and function, contributes to the supply of endoderm in the median ventral stolon. *Doliolum*, in my opinion, represents the most primitive existing instance of this transformation of the epicardial tubes; in this form the epicardial tubes, although both included in the *stolo prolifer*, still retain their primitively paired condition. *Clavelina*, on the other hand, seems to me to represent a further advance in evolution; for here the epicardial tubes, although separate in their origin, unite at an early period to form an unpaired tube. Lastly, the final stage in the transformation of the epicardial tubes is found in *Pyrosoma* and *Salpa*, in which forms the tubes have lost all trace of their primitively paired origin, and arise as a median diverticulum of the pharynx, in the region of the posterior *cul-de-sac* of the endostyle.

It will be conceded, I think, that these modifications of the epicardial tubes provide a sound basis for a true and genetic classification of Tunicate budding; and the subjoined scheme is founded upon this basis. It should be premised, however, that the first group within the scheme is marked off from the rest by the entire absence of special epicardial outgrowths. Budding in this group is effected in a much less specialised manner than in any of the others; in it the endoderm of the bud is not derived from a single source but from several, by the separate origin and eventual union of pharyngeal and gastric portions. Without entering into a special description of this type of budding—for which

reference should be made to the works of Della Valle—it may be remarked that the budding of the Didemnidæ and Diplosomidæ presents features which render it probably the most primitive of all the types found in the Tunicata.

Type 1. *Œsophageo-atrial.*—Endoderm of bud derived both from the œsophagus and atrium (peribranchial cavity) of parent. Didemnidæ, Diplosomidæ.

Type 2. *Atrial.*—Endoderm of bud derived from atrium of parent. Buds lateral and paired, *e.g.*, Botryllidæ, Polystyelidæ.

Type 3. *Pharyngeal.*—Endoderm of bud derived by means of tubular (epicardial) diverticula from the pharynx of parent. Buds unpaired, median.

(i.) Epicardial tubes paired, not united, but only one functional, *e.g.*, ventral bud of larval *Distaplia.*

(ii.) Epicardial tubes sooner or later united distally to form an unpaired tube.

(*a*) Independent in parent, but uniting secondarily in bud, *e.g.*, *Doliolum.*

(*b*) Uniting in parent, but retaining the double communication with pharynx, *e.g.*, *Clavelina*, Distomidæ, Polyclinidæ, *Perophora* (?).

(iii.) Epicardial tubes no longer paired, but developed from the first as a single median posterior prolongation of endostyle, *e.g.*, *Pyrosomidæ*, *Salpidæ.*

It is interesting to compare the above classification, which is founded upon structural characters, with one based on the position of the buds with regard to the parent. It will at once be seen that the two schemes correspond very closely with each other, and as the second scheme may not be without some usefulness, I give it below.

Group 1. Buds lateral, on one side only. Didemnidæ, Diplosomidæ.

Group 2. Buds lateral, on one side of larva, but on both sides of buds. Botryllidæ, Polystyelidæ.

Group 3. Buds ventro-lateral, but only formed on one side. Larval *Distaplia.*

Group 4. Buds median and ventral. Thaliacea.

(i.) Minute. Doliolidæ.

(ii.) Large. Pyrosomidæ, Salpidæ.

Group 5. Buds median and posterior.

(i.) Permanently connected with parent. *Clavelina*, *Perophora*.

(ii.) Detached from parent.

(*a*) Formed from simple stolon. *Distaplia*.

(*b*) Formed from post-abdomen. Polyclinidæ.

4. *The Origin of Budding*.—If I have been at all successful in my analysis of the various types of budding in Tunicata, and in my treatment of the evolution of the epicardial tubes, it must be clear to the readers of this article that the existing types of budding represent merely the final stages of a long process of evolutionary change. Upon first considerations one is tempted to regard the simple process of budding, which I have called undifferentiate, as more primitive than the differentiate. In the undifferentiate buds of *Clavelina* and other forms the whole process of organogeny is strikingly similar to the growth of an embryo from the gastrula stage; and this analogy inclines one at first to believe that the simple structure of the undifferentiate bud has the same primitive significance as that of the gastrula. The phylogeny of budding, however, cannot be determined by the study of the bud alone; it is necessary to consider also the associated changes which have taken place in the structure of the parent form.

Attention to this consideration reveals the fact that the primitive character which one is inclined to ascribe to the triploblastic bud of *Clavelina*, on account of its simple structure, is not borne out by the character of the associated structures of the parent form. We have already seen that the epicardial tube of *Clavelina* is not a primitive structure, but results from the fusion of a pair of tubes; that median budding was preceded phylogenetically by lateral budding; and that the lateral buds of *Botryllus* have a more differentiate character than the median stolonial buds of *Clavelina*.

Another point in this phylogenetic history must be mentioned. In the larva of *Distaplia* two epicardial tubes are present, but one only is functional. In *Botryllus*, also,

the larva gives rise to a bud on only one side of the body (the right side); and, although the buds of successive generations of buds are usually paired, there is always a certain asymmetry in their development: the buds on the right side being always further advanced than those on the left (Oka). The asymmetry which characterises budding in these more primitive cases seems to me to have considerable significance, and to point to the conclusion that the power of bud-production was originally restricted to one side of the body; in other words, that the production of twin-embryos or twin-zooids which characterises the Didemnidæ and Diplosomidæ is a primitive phenomenon.

Now Salensky's recent work (26, 27) upon the development of the twin-embryo of *Diplosoma* shows conclusively that this is a true case of embryonic fission, although the process is condensed and modified in various particulars.

The conclusion, therefore, is inevitable that all the types of budding in the Tunicata are ultimately referable to an ancestral process of embryonic longitudinal fission, as Balfour and Uljanin have already suggested. We may also conclude that budding has probably not arisen more than once within the group of Tunicata, since the various types of asexual reproduction found within it form a single phylogenetic series, and are connected with one another by numerous gradations; and that while the differentiate type of budding is probably in many respects more primitive than the undifferentiate type, yet that there have been modifications in both directions since budding first arose, leading in some cases to a secondary simplification of the organisation of the bud, and in others to a slightly increased complexity.

BIBLIOGRAPHY.

(1) BALFOUR, F. M. *Comparative Embryology*, vol. ii.

(2) BARROIS, JULES. Recherches sur le Cycle Génétique et le Bourgeonnement de l'Anchinie. *Journ. de l'Anat. et de la Physiol.*, xxi., p. 193, 1885.

(3) BENEDEN, ED. VAN, et JULIN, CH. Recherches sur le Morphologie des Tuniciers. *Arch. de Biol.*, vi., 1887.

(4) BROOKS, W. K. *The Genus Salpa.* Baltimore, 1893.
(5) DELLA VALLE, A. Nuove contribuzione alla Storia naturale delle Ascidie composte del Golfo di Napoli. *Mem. Acc. Lincei* (3), x., 1881.
(6) DELLA VALLE, A. Sur le bourgeonnement des Didemnides et Botryllidés. *Arch. Ital. Biol.*, vii., p. 50, 1882.
(7) FOL, H. Etudes sur les Appendiculaires du détroit de Messine. *Genève*, xxi., 1872.
(8) GARSTANG, W. On the Development of the Stigmata in Ascidians. *Proc. Roy. Soc.*, li., pp. 505-513, 1892.
(8A) GIARD, A. Recherches sur les Synascidies. *Arch. Zool. Expér.*, i., 1872.
(8B) GROBBEN, C. Doliolum und sein Generationswechsel. *Arb. Zool. Inst. Wien*, iv., 1882.
(9) HJORT, J. Ueber den Entwicklungscyclus der zusammengesetzten Ascidien. *Mitth. Zool. Stat. Neapel*, x., 1893.
(10) HJORT, J. Beitrag zur Keimblätterlehre und Entwicklungsmechanik der Ascidienknospung. *Anat. Anz.*, x., p. 215, 1894.
(11) JOLIET, L. Études anatomiques et embryogéniques sur le Pyrosoma giganteum. Paris, 1888.
(12) KOROTNEFF, A. La Dolchinia mirabilis. *Mitth. Zool. Stat. Neapel*, x., 1891.
(13) KOROTNEFF, A. Tunicatenstudien. Knospung des Keimstockes bei den Salpen. *Mitth. Zool. Stat. Neapel*, xi., p. 325, taf. xv., 1894.
(14) KOROTNEFF, A. Embryonale Entwicklung der Salpa democratica. *Biol. Centr.*, xiv.. p. 841, 1894.
(15) KORSCHELT und HEIDER. *Lehrbuch der vergl. Entwick. der wirbellosen Thiere*, iii. Jena, 1893.
(16) KOWALEVSKY, A. Ueber die Knospung der Ascidien. *Arch. f. mikr. Anat.*, x., 1874.
(17) LAHILLE, F. *Recherches sur les Tuniciers.* Toulouse, 1890.
(18) LANG, ALBERT. *Zeit. f. wiss. Zool.*, liv., p. 365, 1892.
WEISMANN. *The Germ-plasm*, pp. 155, 156, 1893.
(19) OKA, A. Ueber die Knospung der Botrylliden. *Zeit. f. wiss. Zool.*, liv., 1892.
(20) PIZON, A. Histoire de la Blastogénese chez les Botryllidés. *Ann. des Sci. Nat.* (8), xiv., p. 1, 1893.
(21) RITTER, W. E. On Budding in Goodsiria and Perophora. *Anat. Anz.*, x., p. 364, 1895.
(22) SALENSKY, W. Ueber die Knospung der Salpen. *Morph. Jahrb.*, iii., 1877.
(23) SALENSKY, W. Neue Untersuchungen über die embryonale entwickelung der Salpen. *Mitth. Zool. Stat. Neapel*, iv., 1883.

(24) SALENSKY, W. Beiträge zur Entwicklungsgeschichte der Pyrosomen. *Zool. Jahrb., Abth. f. Anat.*, iv., 1891, v., 1892.

(25) SALENSKY, W. Ueber die Metamorphose der Distaplia magnilarva. *Morph. Jahrb.*, xx., p. 449, 1893.

(26) SALENSKY, W. Ueber die Entstehung der Metagenesis bei Tunicaten. *Biol. Centr.*, xiii., p. 126, 1893.

(27) SALENSKY, W. Ueber die Entwicklung von Diplosoma Listeri. *Mitth. Zool. Stat. Neapel*, xi., 1894.

(28) SEELIGER, O. Ueber das Verhalten der Keimblätter bei der Knospung der Cölenteraten. *Zeit. f. wiss. Zool.*, lviii., p. 152, 1894.

(29) SEELIGER, O. Zur Entwicklungsgeschichte der Pyrosomen. *Jenaische Zeit. f. Naturwiss.*, xxiii., 1889.

(30) SEELIGER, O. Ueber die Entstehung des Peribranchialraumes in den Embryonen der Ascidien. *Zeit. f. wiss. Zool.*, lvi., p. 365, 1893.

(31) ULJANIN, B. Doliolum. *Fauna und Flora des Golfes von Neapel*, 1884.

(32) WILLEY, A. Studies on the Protochordata. *Quart. Jour. Micr. Sci.*, xxxiv., p. 338.

WALTER GARSTANG.

THE RESERVE MATERIALS OF PLANTS.

(*Continued.*)

IN addition to the nitrogenous matters already described as occurring in plants, a curious body of proteid character can be extracted from the ground seeds of several of the cereal grasses, the exact nature of which has given rise to a good deal of controversy. This body, which is known as *gluten*, can be prepared easily from wheat flour by washing it on a muslin filter. The starchy matters and various soluble bodies pass through; a curious sticky material is left clinging to the muslin. No such proteid is found to be present in the uninjured seed, and it appears therefore to be formed during the process of washing the flour.

Investigations into its nature and its relation to the proteids of the wheat grain have been made by several writers, and a leaning is found to the view that it is a kind of vegetable fibrin, produced by a species of fermentation analogous to that so generally associated with the process of the coagulation of the blood. In the light of this discussion considerable interest attaches to some investigations into the proteids of the seeds of the cereal grasses, particularly wheat and rye, carried out last year in the laboratory of Professor Chittenden, by Messrs. Osborne and Voorhees (19).

These observers have made a careful analysis of the constituents of the uninjured flour, in which they have confirmed and extended the researches of older writers. They find the wheat grain to contain five distinct proteids, of which three can be classified as members of the already well-known groups, while two others have special peculiarities as to solubility in fluids which do not attack the former three. The latter are a globulin, an albumin and a proteose.

The globulin, which the authors name *Edestin*, is a member of the vitellin section of the group, characterised

by being soluble in saturated solutions of sodium chloride. Its globulin character is established by the fact that it is insoluble in water, but has the power of dissolving in weak saline solutions. It can be precipitated from the latter by large dilution or by dialysis; also by saturation of the solution by magnesic sulphate or ammonic sulphate. It shows a peculiarity in its relation to heat, being *precipitated* on boiling its solution, but in not being at that temperature converted into coagulated proteid. Wheat flour contains about ·6 to ·7 per cent. of edestin.

The albumin has been named *leucosin;* it is present in less quantity than edestin, about ·3 to ·4 per cent. being obtainable from the flour. Its reactions show certain peculiarities; it is, unlike most albumins, precipitated by saturating its solutions with sodic chloride or magnesic sulphate. On the other hand, it is soluble in distilled water, and has a definite coagulating point, the change taking place at 52°C. This is a much lower temperature than is found necessary to coagulate the animal albumins, which remain unchanged up to about 70 to 80°C. It can be separated from edestin by dialysis, which throws down the latter, but leaves the leucosin in solution.

The proteose is perhaps not properly included as a reserve proteid of the grain. As we shall see changes take place in the wheat kernel in consequence of keeping after maturity, so that this body may be rather one on its way to such a condition as will enable it to be transported from the seed, thus forming a link with the circulating proteids and not a truly reserve product. The proteoses are as a rule the first products of the action of a digestive ferment.

This body, whatever be its actual position in this respect, can be isolated from wheat flour, freshly ground, and extracted with dilute saline solution. After such an extract has been dialysed till the edestin is all precipitated, and subsequently boiled to remove the leucosin, it still gives proteid reactions. Saturation with sodic chloride, or the addition of about 20 per cent. of the same salt after acidulating the solution with a little acetic acid, throws down the

proteose. It is not coagulated by heating to boiling point, but if the temperature be maintained at that point till the solution becomes concentrated, a coagulum is gradually developed. There is about ·3 per cent. of this body in the flour.

The other two proteids of the flour are those to which the formation of the gluten can be attributed. They both show a certain resemblance to the *zein* described ("SCIENCE PROGRESS," vol. ii., p. 121) as occurring in the seeds of Zea Mais, in that they are more or less completely soluble in warm dilute alcohol.

The first of them was described under the name of *gliadin* by Tadder in 1820, and was referred to also by Liebig under the name of *plant gelatin*.

Gliadin is the proteid which is present in largest quantity in the seed of wheat, about 4 per cent. of the flour being composed of it. It is present also in rye.

Its preparation is most easily effected by taking advantage of its solubility in hot dilute alcohol. If either wheat or rye flour, or the gluten prepared from them, be treated with this solvent, the gliadin dissolves, the best result being obtained by using a mixture of seventy parts of absolute alcohol with thirty parts of water. From this solution it is precipitated by the addition of sodic chloride. When the precipitate is collected, dehydrated by absolute alcohol, and dried over strong sulphuric acid, it remains as a powdery white mass. If the precipitate is not dehydrated, but moistened with dilute alcohol or water before drying, it is not powdery, but has the appearance of gelatin, being clear and transparent. This recalls the old name which Liebig originally gave to it. Cold water fails to dissolve it with any ease, rendering it extremely viscid and sticky; water gradually warmed has more effect upon it, but even at boiling point it is not all dissolved, and as the temperature subsequently falls part of it separates out again. What is left in solution is partly precipitated by the addition of a little sodic chloride, as is the case with the original alcoholic extract.

Gliadin dissolves readily in dilute acids or alkalies, and

is precipitated on neutralisation of the solution without any alteration; a point indicating a possible relation to the derived albumins of digestion, from which, however, its relation to alcohol separates it. When it is warmed with sulphuric acid of 50 per cent. concentration it slowly assumes a violet colour, which is increased in intensity by boiling. Concentrated hydrochloric acid causes the same coloration.

Though similar to zein in its behaviour with alcohol, the authors consider it not to be identical with the latter.

The remaining proteid of the seeds has been called by the discoverers *glutenin.* It is only slightly soluble in warm alcohol or in warm water, but can be prepared from the flour by treating it with dilute acids or alkalies. If it be precipitated from the solvent, dehydrated with absolute alcohol, and dried over strong sulphuric acid, it dissolves slowly again in the same reagents, but leaves a little coagulated residue. Before the dehydration the precipitate is very readily soluble in either weak potash or dilute hydrochloric acid.

It differs from gliadin in its reaction with 50 per cent. sulphuric acid, turning brown instead of violet; it takes on the latter colour, however, with concentrated hydrochloric acid.

From the general similarity of its reactions to those of gliadin it seems possible that it may be a result of some decomposition of the latter. The discoverers, however, think it is a wholly distinct body, as it cannot be prepared from rye flour, though the latter contains a good deal of gliadin. Glutenin is almost as plentiful in wheat as gliadin, nearly 4 per cent. of the flour being composed of it.

Both gliadin and glutenin being capable of preparation from gluten, as well as from flour, these investigations throw a good deal of light on the constitution of the latter. There appears to be no transformation of its constituents as in the process of the formation of fibrin, nor is any ferment necessary for its production. To form gluten the authors put forward the view that the gliadin and glutenin, both of which exist separately in the wheat endosperm, combine with water in the proportion of one part of the

proteids to two parts of water. There is no change in their character in consequence, but their union is a mechanical one, the gliadin forming a gummy or adhesive mass, and the glutenin affording a basis or framework to which the gliadin adheres. This support enables the gliadin to cause the other parts of the flour to stick together, and thus form the dough which results when the flour and water are kneaded.

The authors do not quote any microchemical experiments, so that the distribution of the several proteids in the seed has not been ascertained.

Before leaving the subject of the proteid reserve materials it is interesting to notice that though their composition may with fair accuracy be ascertained through analysis of them, either in bulk or by microchemical methods, it by no means follows that when once they are deposited in definite reservoirs their composition remains unaltered till they are called upon to take part in the nutrition of the plant. Only a few observations on this point are on record, but these lead to the view that even in their most permanent form they are comparatively unstable, and that a certain metabolism is constantly proceeding in the cells in which they lie, in which they are to a greater or less extent involved. The cells at the time of deposition we have seen to be rich in protoplasm, and we have found that it is at least probable that this protoplasm is concerned in the process of their deposition. In the case of carbohydrates, such as starch, it has been shown by Brown and Morris (20) while the embryo of the barley is making its way into the parenchyma of the endosperm, and while the latter is thus in a stage during which starch deposition in the cells is proceeding, a form of diastase is present in them with their protoplasm. Whether in the lupin and other seeds any such enzyme is present at a corresponding stage has not been ascertained, but there seems little reason to deny that when the influx of material, which is the source of the aleurone grains, has ceased, the needs of the protoplasm may require to be supplied by the aleurone itself. Nor is this hypothesis simply; it can be determined by

comparative analyses taken at considerable intervals of time that the proteids change after deposition. Whether the changes only go on during a comparatively short period, corresponding to the ripening of the seed, or whether they are prolonged till the power of germination is lost, remains an open question.

Some experiments bearing on this point were published by Acton (21) two years ago, analyses having been made of a sample of new wheat, and of another of wheat grown upon the same land thirty years before, during the interval it having been kept in the stack. Too much stress must of course not be laid upon the experiment, as it is probable that the samples did not exactly correspond. The results of the analyses showed that the old wheat had lost nearly half its water; that compared with the new wheat the proteids insoluble in water were as 4·3 : 7·1; those soluble in water 3·9 : 1·2. Asparagin, which was absent from the new wheat, was present in the old sample to the extent of ·8 per cent. The tendency during ripening, or subsequently, was therefore in the direction of forming soluble and diffusible bodies from others which were neither. During the interval the old wheat had lost the power of germinating, and contained no enzyme, though this was present in the new sample.

The nitrogenous reserve materials which are found in form of amides are probably almost entirely confined to the circulating supply. A few are found in resting seeds, but they are more readily detected after germination has begun, and must therefore be looked upon rather as intermediate products. Being as a rule soluble in the cell sap, they are with difficulty detected. Belzung (22) has succeeded by a new method in ascertaining their presence. He places fresh sections of the living seed in strong glycerine; after the lapse of a variable time, ranging from a few hours to two or three days, the amides crystallise out in their characteristic forms. The method is sufficiently accurate to yield a good idea of the relative amounts present. It needs supplementing of course by the extraction of the amides from considerable quantities of the seeds and a sub-

sequent chemical analysis. Belzung describes asparagin as being the form most commonly met with, though leucin and tyrosin occur more frequently than has been supposed, and in greater amount. In germinating seeds of *Cicer arietinum* he detected as a normal product the body *xanthin*, which has hitherto been held to be a product of putrefactive changes where it occurs in plants.

Of carbohydrate reserve materials the form most widely distributed is that of starch. Its occurrence is almost universal, nearly all plants at some time or other forming it, and usually in very large amount. Its places of storage cannot be enumerated, nor can any description of the grains composed of it embrace every form found. Being insoluble in water, and readily identified by its characteristic reaction with iodine, it presents but little difficulty in its detection. Wherever found it appears as grains of definite shape, and these often characteristic each of its own particular plant. The sizes and complexity of structure of the grains vary very much, even in different parts of the same plant.

It has been generally held that the appearance of starch in different parts has a different meaning from the point of view of the metabolism of the plant. In parts not exposed to light, it has always been looked upon as a true reserve material, intended for either immediate or deferred consumption. But in leaves and other chlorophyll-containing parts a different interpretation has been given. Occurring originally in the interior of the chlorophyll grains, and being found almost immediately upon the absorption and decomposition of carbonic dioxide, it has been held to be a stage in the process of construction of organic material; indeed, it is usually described as the first visible product of assimilation.

Some investigations made by Brown and Morris (23) on the metabolic changes in foliage leaves tend to disprove that view, and to lead to the opinion that in leaves as elsewhere starch grains are always the results of a process of destructive metabolism, and are reserve materials, even when occurring in the bodies of chlorophyll grains. The experiments were made with the leaves of the garden

nasturtium *Tropœolum majus*, and took the form of an investigation of the nature of their carbohydrate constituents under various conditions of illumination or insolation. A number of leaves detached from the plants in early morning were divided into two portions; one set were killed at once by heating in a steam chamber; the others were exposed with their petioles in water to sunshine for twelve hours, being spread upon wooden trays covered with wire gauze. They were then killed as were the former set. The contents of the cells showed great difference in their carbohydrates, the starch increasing from 1·23 to 3·91 per cent. of the dry weight, and cane-sugar going up from 4·65 to 8·85 per cent. In another experiment the increase was for starch from 3·24 to 4·22 per cent., and for cane-sugar 4·94 to 8·02 per cent. At the same time the total sugars of the leaf, including lævulose, dextrose and maltose, nearly doubled in amount. The chief feature which thus accompanies assimilation of carbonic dioxide in these leaves is the formation of a great amount of cane-sugar, the absolute quantity being always much greater than that of the starch. The authors conclude that when assimilation is vigorous this body is made to accumulate much faster than it is used, and the leaves are consequently charged with it, possessing therein a temporary reserve material of carbohydrate. As soon as the concentration of this sugar in the cell-sap and protoplasm exceeds a certain strength, starch commences to be elaborated by the chloroplasts, forming a more stable and permanent reserve material than the cane-sugar. The smaller increase of the starch as compared with that of the cane-sugar is intelligible on the hypothesis that the latter, and not the former, is the first constructed carbohydrate, and that the starch is formed as a secondary product from it, as in the case of underground deposits it is produced by the leucoplasts from another sugar transported to them.

On reviewing these experiments it at first seems strange that cane-sugar should be the immediate antecedent of starch, the latter being so much nearer maltose in its constitution. The conclusions are strengthened, however, by

the experiments on the artificial nutrition of leaves by solutions of carbohydrates, published by A. Meyer in 1886 (24), and by Laurent in the same year (25), and by those of Acton (26) on the absorption under abnormal conditions of organic compounds by the roots of various culture plants. All these observers have shown that when the only source of carbon that was afforded to the plants with which they experimented was a weak solution of cane-sugar, the latter was gradually absorbed, and in due course starch made its appearance in the cells of the parenchyma of the leaves. The experiments of Brown and Morris on the artificial feeding of isolated barley embryos (29) with solutions of cane-sugar also support the view that the latter may readily give rise to starch. In these experiments excised embryos were deprived of starch by an exhaustive water culture of five or six days, and then placed upon dilute solutions of cane-sugar, and starch was speedily formed in the cells of the scutellum.

Since Schimper's classical researches upon the mode of formation of starch grains, their appearance has been attributed uniformly to the action of plastids, either leucoplasts, as in parts not exposed to light, or chloroplasts, in the illuminated regions. The striation of the larger grains has consequently been held to be due to apposition of successive layers upon the surface of the grains. Recent writers claim, however, that this view is insufficient, and that probably in many cases the deposition is effected by the immediate action of the protoplasm of the cell.

The leucoplast theory is undoubtedly a good deal strained by the cases where immense numbers of very small grains, hardly larger than mere specks, make their appearance in rapidly growing structures, particularly as even high powers of the microscope fail to show the presence of any plastid before or during the deposition. In the pollen grains of many plants before their germination, and in the pollen tube as it develops, small specks of starch make their appearance. In the pollen of *Zamia skinneri* the ungerminated grain shows a fairly homogeneous protoplasm, scarcely even granular. When this

pollen is placed in a nutritive fluid (27) very speedily small granules of starch appear in this homogeneous protoplasm, and the number continually increases as germination begins. In the pollen tubes of the lily the same thing is seen, the number of small grains being enormous. In the style of the same plant, in the cells containing again quite transparent protoplasm, the same formation is found, the cells in some cases becoming quite filled with starch. It is rather hard to conceive in these cases of a simultaneous formation of plastids to carry out the deposition.

The same considerations apply to the deposition of starch grains in young developing embryos. Some observations, published by Belzung (28), made upon the young embryos of various Leguminosæ, particularly *Lupinus albus* and *Phaseolus vulgaris*, point to the non-occurrence of the leucoplast as an antecedent to starch deposition. Indeed, Belzung goes so far as to advance the view, in opposition to Schimper, that the leucoplast theory is based upon an erroneous interpretation of the appearances seen.

His conclusions may be briefly stated as follows: In *Lupinus albus* and *L. mutabilis* there are no plastids in the tissue of the developing embryo, yet starch is deposited there during the first phase of its development, which starch later disappears during the ripening of the seed. To a less extent, the same thing may be observed in the embryo of *Phaseolus vulgaris*. The protoplasm of the cells of such embryos in course of formation may be seen to have the form of a network with many small meshes, which are empty spaces, or contain only cell-sap. There is no leucoplast inside them, nor anything comparable to one. The starch grains originate in these meshes at some point in contact with the protoplasm, and gradually increase in size till they fill them, growing in fact like crystals, and forming the *simple* grains of starch. Later these disappear, and in cases where starch appears further on in the development in the form of *compound* grains, the formation is due to the vacuole or mesh being partly filled by a smaller and much finer network, giving still smaller meshes. In these smaller meshes, forming part of the larger original

one, starch granules appear as before, and, growing, become pressed together till the whole vacuole is full. The new grains appear consequently compound on account of the new fine network which separated the seats of commencement of formation of the grains. Belzung concludes that Schimper's leucoplasts are nothing more than cavities in the protoplasm, which become filled with starch by the action of the bounding layer of the former.

Schimper states that the grains which are situated deep in the chloroplasts have a concentric striation, while those which are formed near their surface are excentrically striated, and he attributes the phenomena to the relation of the grain to the substance of the plastid. Belzung contends, on the contrary, that while the starch grain is in the chloroplast no striation can be seen, but after it escapes from the plastid it becomes striated. The escape of the starch grain from the latter can be seen best in those cases where its origin is near the surface of the plastid, and not in its centre.

Eberdt (29) partly supports Belzung's views, but puts forward a new interpretation of the appearance of the leucoplast. He says that the growth of an excentrically striated starch grain is not due to the action of any unilaterally attached body such as a plastid; both the excentric and the concentric ones can be seen to grow in the cell-sap by the action of the protoplasm. In the case of the grains formed in connection with the chloroplasts he observes the same thing, the grain growing after the chloroplast has disappeared, and being nourished or added to by the protoplasm, wherever the latter is in contact with it. He suggests that the development of the starch grain has two phases, it being originated by the chloroplast, and then later built up by the protoplasm. The striation he holds to be due to intussusception, and not to apposition at all.

In the formation of the starch grains in relation to leucoplasts Eberdt thinks the latter is not a plastid in the sense of constructing the grain, but is rather a formative material or fundamental substance which becomes transformed into the substance of the starch grain. The so-

called leucoplast being smaller than the latter constitutes a nucleus of formation, and the grain so started subsequently grows and becomes differentiated into layers. He speaks of the protoplasm at first as filling the younger cells and being finely granular. A little later the granulations are larger, having increased at the expense of the homogeneous part of the protoplasm. These larger granulations when stained with iodine show in their interior a reddish point, which at a slightly later stage becomes a blue one. A little later the granulations stain blue throughout, and are evidently starch grains. They are often united into groups of two, or three, or more, and are surrounded by protoplasmic particles.

Eberdt thus argues for the appearance of the leucoplast, but denies its *plastid* nature, holding it to be a fundamental substance, on its way to become starch.

He denies that the leucoplast multiplies by division, and holds that each one found is newly and separately differentiated out of the protoplasm.

Konnigsberger (30) advances again a different view to the last two writers, and enables us partly to reconcile the differences which their views present from those of Schimper and the other writers of his school. According to him some plants contain leucoplasts and others do not. His work extends over thirty species—eighteen monocotyledons and twelve dicotyledons. He finds leucoplasts in twelve monocotyledons and four dicotyledons, the other species examined being without them. He differs from Eberdt with regard to the so-called fundamental substance, which Eberdt thought to be such on account of the red stain with iodine which preceded the appearance of the blue. He explains the difference of tint as being an illusion due to the small size of the staining body, and holds that we have a leucoplast in which starch originates. Unlike Schimper, he finds excentrically striated grains deeply seated in chloroplasts, in the tissues of *Pelargonium* and of *Canna indica*, and thinks that excentricity of striation is not due to lateral deposition. The lateral deposition hypothesis he finds contradicted by the fact that in some

grains he observed that the larger side of the grain is *opposite* to the position of the leucoplast, instead of being in contact with it. In *Maranta arundinacea* he observed cases where the starch grains originated in contact with a leucoplast, but subsequently became free from it, and afterwards grew while embedded in the protoplasm.

It appears therefore most probable that the importance of the plastid has been somewhat exaggerated in connection with the development of starch grains, and that although its influence is very generally exerted, the protoplasm itself is capable of forming the starch grain as it is other constituents of the ordinary cell contents.

Another point of some interest arises in connection with the plastids, whether coloured or not. It has been generally held that they are not differentiated anew from the protoplasm at the seat of their appearance, but are derived from others by ordinary fission. On this hypothesis one at least must be present in the fertilised egg-cell. No such structure appears in the pollen grain, or the pollen tube, and hence all the plastids of a plant must be derived from its female parent. This seems an anomaly difficult to explain.

Glycogen as a reserve material is especially characteristic of fungi, in several of which it has been detected. Usually the reserves in fungi are but slight, and take various forms, especially oil, but sometimes cellulose and glycogen, the latter occurring with the other two or one of them. The reserve materials of fungi have chiefly been studied in connection with the sclerotia, particularly in the case of ergot (*Claviceps purpurea*). In the sclerotia described by Errera (31) as *Sclerotium stipitatum*, a mass of tissue exists consisting of small thin-walled, rounded cells mixed with long fibres, whose cavities are nearly obliterated. The former are filled with glycogen. When the sclerotia germinate the glycogen diminishes in amount, and in some form or other passes into the new hypha. The form in which it travels has not been ascertained, but it is probably a form of sugar as in other carbohydrate migrations. In the young hyphæ temporary accumulations or transitory re-

serves may be found deposited in the form of granular masses, which stain reddish with iodine. According to Errera the reserve oil of the fungus can give rise to similar formations. The mode of this transformation must, however, be very different. The deposition of these grains of glycogen has also been studied by Belzung (32), who has seen the process in the hyphæ of several species. He describes them as starch grains of small size, rather than glycogen. As, however, his opinion of their nature is derived from staining them with iodine, too much stress need not be laid upon this point, particularly as in a later paper he says that some of them are coloured brown by the reagent.

He describes their deposition as associated with the presence of certain corpuscular bodies which themselves are stained brown with iodine. These he conjectures to be of the same nature as the chlorophyll bodies of the higher plants, on the origin and nature of which he holds peculiar views. Whatever be their origin they appear to function in the way of Schimper's leucoplasts, and to deposit the carbohydrate material, whether glycogen or starch, in definite granular form.

In these plants the consumption of the reserve carbohydrate takes place particularly during the development of the perithecia and the formation of the spores. The latter do not, however, contain any glycogen, their reserves taking the form of oil. On their germination, however, as the oil disappears the young hyphæ are often found to contain transitory glycogen, much as in the germination of oily seeds the young tissues contain transitory starch.

Though the details of the transformations are obscure, there seems to be little doubt that the two bodies are intimately connected with one another. Also that in the fungus the glycogen takes the place occupied in the green plant by starch.

BIBLIOGRAPHY.

(19) OSBORNE and VOORHEES. Proteids of the Wheat Kernel. *Journal of the American Chemical Society*, xvi., No. 8, Aug., 1894.

(20) BROWN and MORRIS. On the Germination of some of the Gramineæ. *Journal of the Chemical Society*, vol. lvii., June, 1890.

(21) ACTON, E. H. Changes in the Reserve Materials of Wheat on Keeping. *Annals of Botany*, vii., Sept., 1893.

(22) BELZUNG. Sur divers principes issus de la germination. *Journal de Botanique*, t. vi., 1892.

(23) BROWN and MORRIS. A Contribution to the Chemistry and Physiology of Foliage Leaves. *Journal of the Chemical Society*, May, 1893.

(24) MEYER, A. Bildung der Stärkekörner in den Laubblättern aus Zuckerarten, Mannit, und Glycerin. *Bot. Zeit.*, Nos. 5-8, 1886.

(25) LAURENT. *Bot. Zeit.*, p. 151, 1886.

(26) ACTON. The Assimilation of Carbon by Green Plants from certain Organic Compounds. *Proc. Roy. Soc.*, p. 150, 1890.

(27) GREEN. On the Germination of the Pollen Grain and the Nutrition of the Pollen Tube. *Phil. Trans.*, 185 b., 1894.

(28) BELZUNG. Recherches morph. et physiol. sur l'amidon et les graines de chlorophylle. *Ann. des Sc. Nat.*, ser. vii., t. v.

(29) EBERDT. Beiträge zur Entstehungs geschichte der Stärke. *Jahrb. fur. wiss. Bot.*, bd. xxii., heft 3.

(30) KONNIGSBERGER. Recherches sur la formation de l'amidon chez les Angiospermes. *Archives Néerlandaises des Sciences exactes et Naturelles*, t. xxvi., pp. 217-258.

(31) ERRERA. Les reserves hydrocarbonees des champignons. *Comptes Rendus*, Aug. 3, 1885.

(32) BELZUNG. Recherches sur l'ergot du Siegle. *Ann. des Sc. Nat.*, ser. vii., t. v., p. 288.

J. REYNOLDS GREEN.

(*To be continued.*)

PULSE TRACINGS FROM MR. CONWAY'S PARTY, 1892.

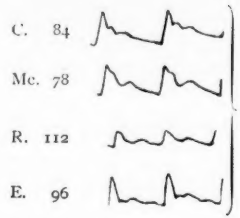

FIG. 1. 23rd April, 2 p.m. Burzil Kote, about 12,000 feet. A day of rest in camp in miserable weather; snowing.

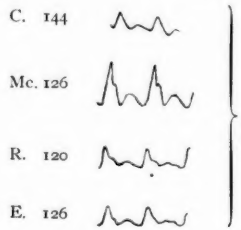

FIG. 2. 24th April, 2 p.m. Burzil (or Dorikun) Pass, about 14,000 feet. Tracing taken three quarters of an hour after reaching the Col, after five hours of laborious ascent in deep snow. Several of the servants badly affected to-day, who gave no signs of suffering at much higher altitudes later on. All of us probably in poor condition.

FIG. 3. 15th August, 10.30 a.m. In a snowstorm. We were resting in the tent all day at Junction (Throne glacier) Camp, about 16,000 feet.

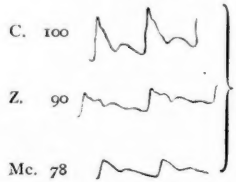

FIG. 4. 19th August, 7.30 p.m. After a day of rest at Footstool Camp, about 17,000 feet.

FIG. 5. 21st August, 11.30 a.m. Quarter of an hour above Serac Camp, about 18,000 feet. After a morning's severe climbing. Fine weather.

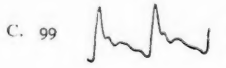

FIG. 6. 22nd August, 6.40 a.m. Serac Camp, about 18,000 feet. After a night's rest. Fine weather.

FIG. 7. 23rd August, 2.15 p.m. Lower Plateau Camp, about 19,000 feet. After a morning's climb, followed by several hours' rest. Fine weather.

FIG. 8. 24th August, 10.30 a.m. Upper Plateau Camp, about 20,000 feet. After a morning's climb (when we were nearly frost-bitten), and two hours' rest.

FIG. 9. 25th August, 3 p.m. Top of Pioneer Peak, about 23,000 feet. After eight hours of climbing, with a few rests.

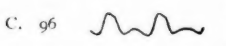

FIG. 10. 25th August, 4.30 p.m. Water Col, close to second summit, about 22,000 feet. After thirty-five minutes' descent from highest peak.

FIG. 11. 27th August, noon. At Footstool Camp (see Fig. 4). During a day's rest and bad weather.

FIG. 12. 1st September, 7.30 p.m. At Corner Camp, about 13,000 feet. After a day's rest.